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ARTICLES

Full Length Research Paper

Vitamins C and E levels are enhanced by *Azadirachta Indica* leaves aqueous extract in paracetamol induced hepatoxicity in Wistar rats

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Azadirachta indica, used in many parts of the world as herbal medicine, has been reported to be antioxidative and hepatoprotective. This study investigated the effect of leaves aqueous extract of *A. indica* on plasma levels of vitamins C and E, liver enzymes alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in Wistar rats with hepatotoxicity. Four groups of twenty Wistar rats each was used. Group A was given normal saline, group B 800 mg/kg body weight of paracetamol, group C 800 mg/kg body weight paracetamol and 400 mg/kg body weight of leaves extract of *A. indica* and group D 800 mg/kg body weight paracetamol and 1000 mg/kg of leaves extract of *A. indica*. The animals were weighed before and after the experiment and the levels of ALT, AST, ALP and vitamins C and E were estimated. There were significant differences between the initial and final mean weights of the animals ($\rho < 0.05$). Plasma liver enzymes were significantly increased in group B, while these enzymes were significantly decreased in group D when compared with B ($\rho < 0.05$). There was no change in ALP levels ($\rho > 0.05$). Vitamins C and E in liver homogenate were decreased in group B and increased in group D ($\rho < 0.05$) while group C showed no change ($\rho > 0.05$). Vitamins C and E were decreased in group B and increased in group D ($\rho < 0.05$). The observed decrease in liver enzymes and increase in vitamins C and E in group D suggest that the extract enhances vitamins C and E levels, and may be hepatoprotective.

Key words: *Azadirachta indica*, hepatotoxicity, vitamins C and E, paracetamol, Wistar rats.

INTRODUCTION

Several pharmacological activities and medicinal application of various parts of *Azadirachta indica* A. Juss (*Meliaceae*) are well known and the biological activities of its leaves aqueous extract have been reported (Pennington and Styles, 1975). According to Boeke et al. (2004), the medicinal potential of the extracts of *A. indica*

can be preventive, curative and/or protective. The chemical constituents found in the leaves of neem are nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-sdesacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione and nimbiol (Hossain et al., 2013). The leaves aqueous extract of *A. indica* has many bioactive components which are useful in the treatment of human diseases. It possesses potent immunostimulant activity which is evidenced by both humoral and cell-mediated responses (Sen et al., 1992). The hepatocellular activity of leaves aqueous extract of *A. indica* has demonstrated that it offers protection against paracetamol induced liver necrosis in rats (Bhanwra et al., 2000). This cellular damage could be a result of oxidative stress, which is caused by an imbalance between the production of reactive oxygen and biological system's ability to readily detoxify the reactive intermediates or easily repair resulting damage (Ihim et al., 2013). The free radicals and reactive oxygen species (ROS) attack lipids, proteins, carbohydrates, and DNA to induce oxidation, cleavage, cross-linking, and modification which eventually cause cell damage (Halliwell and Glutteridge, 1989).

The attack by these free radicals leads to changes in membrane permeability, membrane lipid bilayer disruption and functional modification of various cellular proteins (Valko et al., 2007; Molavi and Mehta, 2004). Reactive oxygen species cause cellular damage leading to many diseases, including cancer, autoimmune disease, and immunodegenerative disease. Such toxic insults are normally detoxified by phase II detoxification enzymes and antioxidant proteins. These antioxidant proteins are modulated by nuclear factor (Nrf2) (erythroid-derived 2)-like 2). Nrf2 is a potent protein and transcription factor that turns on and off the genes that produce antioxidants. It assists in protecting the liver through increased sensitivity to acetaminophen-induced hepatocellular necrosis and hepatotoxicity (Thomson, 2013).

The elevated levels of liver enzymes, indicative of liver damage, were found to be significantly reduced on the administration of *A. indica* leaves aqueous extract in rats (Biswas et al., 2002). The commonest enzymes regarded as indicators of liver damage are aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Hepatic cell damage results in the increase of these enzyme activities (Alimba et al., 2012). Livers of paracetamol-induced stress rats were normal in appearance and histology after the administration of leaves aqueous extract of *A. indica* (Biswas et al., 2002). The extract was observed to cause a reduction of paracetamol induced high serum levels of transaminases (AST and ALT) as reported by Bhanwra et al. (2000).

Vitamins C and E are chain breaking antioxidants and could individually halt the chain of oxidative reactions that ultimately lead to pathology (Niki, 1991). Vitamin C is a water-soluble antioxidant that reacts rapidly with superoxide and peroxyl radicals, and even more rapidly with hydroxyl radicals to give semi dehydroascorbate (Rao et al., 2005). Vitamin C acts as the primary defence in the blood against aqueous radical attack (Frie et al., 1988). The use of these vitamins combined with moderate exercise has been shown to counteract oxidative stress and also lower the level of malondialdehyde (MDA), a critical marker of oxidative stress (Nwanjo and Orjiako, 2006; Kutlu et al., 2005). Antioxidant defences are classified into three groups namely, (i) the preventive antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase(GPx) and metal chelating proteins; (ii) the radical-scavenging antioxidant,such as vitamins C and E, and (iii) the repairs and *de novo* enzymes, such as lipase and DNA repair enzymes (Willcox et al., 2004).

Vitamin E is the most important lipid soluble chain breaking natural antioxidant in mammalian cells and is able to cross the blood-brain barrier and accumulate at therapeutic levels in the brain, where it reduces lipid peroxidation (Veinbergs and Mallory, 2000). It blocks the production of ROS when fats undergo oxidation (Ha et al., 2010). Vitamin E levels also negatively correlate with the production of oxidative stress products and indirectly correlate with the extent of liver damage (Masalkar and Abhang, 2005). Vitamin C or E alone or in combination can facilitate scavenging of free radicals generated in liver tissues (Zaidi et al., 2005).

This study aimed to elucidate the role of *A. indica* leaves aqueous extract in potentiating the antioxidative activity of vitamins C and E using paracetamol induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

The experimental design and laboratory techniques for this study were approved by the Research Ethics Committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus.

Plant materials

Procurement

Fresh matured leaves of *A. indica* were obtained from a local *neem* tree in Ihiala, Anambra State, Nigeria, and identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria.

Extract preparation

The leaves were thoroughly washed and dried in carbolated moisture extraction drying oven (Grant instruments, Cambridge, England) at 45 – 50°C for 3 h. Grinding was done using Thomas contact Mills (PyUnicam, Cambridge, England). The powder was sieved through 1 mm sieve and 200 g soaked in 1000 ml of water and allowed to stand for 48 h. The extract was filtered and the filtrate dried using a hot air oven (Grant instrument, Cambridge,

Table 1. Experimental design showing the groups of Wistar rats and the treatments they were given.

Group	Treatment given
Group A (Control)	Received only normal saline (0.9% NaCl w/v) 5 ml/kg body weight.
Group B	Received only paracetamol (800 mg/kg body weight) once daily.
Group C	Received paracetamol (800 mg/kg body weight) and *A. indica* leaves aqueous extract (500 mg/kg body weight) once daily.
Group D	Received paracetamol (800 mg/kg body weight) and *A. indica* leaves aqueous extract (1000mg/Kg body weight) once daily.

England) at 45-50°C.The residue yield was 52 g and appropriate concentrations made for the experimental design using distilled water (Nunomura et al., 2006).

Paracetamol

Paracetamol tablets (manufactured by Emzor Pharmaceuticals Nigeria Limited) were purchased from a registered pharmacy shop in Ihiala, Anambra State, Nigeria. The tablets were dissolved in distilled water according to the required concentration (w/v) for the administration to the Wistar rats on the basis of body weight.

Experimental animals

Wistar rats weighing 150 – 250 g were procured from the Animal House of College of Medicine and Health Science, Imo State University, Owerri. They were maintained under controlled conditions of light (12/24 h) and temperature. The animals were fed with standard pellet diet (product of Pfizer, Nigeria Ltd) and allowed free access to water *ad libitum* throughout the period of the experiment (Challopadhyay and Bandyopadhyay, 2005).

Experimental design

Eighty Wistar rats were used in this study. They were randomly divided into four groups of twenty animals each and given different treatments as shown in Table 1. Leaves extract, paracetamol, and normal saline were administered with the aid of a feeding cannula.

Sample collection

After 14 days of treatment, all the animals were weighed and sacrificed via euthanasia using chloroform after a fasting of 16 h following the last administration. Blood was collected via cardiac puncture, allowed to clot and then centrifuged at 10,000 revolutions per minute for 5 min using Wisperfuge model 1384 (Tamson, Holland). Serum was separated for various biochemical analyses and stored at -20°C prior to use. The livers were dissected from all the animals, cleared of blood using normal saline and immediately transferred into blood ice-cold container of normal saline. The livers were homogenized in 0.1 N tris-HCL buffer (7.4) and used for the estimation of vitamins C and E (Challopadhyay and Bandyopadhyay, 2005).

Acute toxicity testing

The acute toxicity of *A. indica* leaves aqueous extract was done using 30 mice divided into 5 groups of 6 mice each. Each group received graded doses (200 – 1000 mg/kg body weight) of the extract and the animals observed for toxic effects after 48 h of treatment. The toxicological effect was observed in terms of mortality expressed as LD_{50}. The number of animals that died during the period was noted. The LD_{50} of the extract was estimated from the graph of percentage (%) mortality, converted to probity, against log-dose of the extract, probit 5 being 50% (Litch and Wilcoxon, 1959).

Laboratory methods and procedures/biochemical analysis

All reagents were commercially procured and with strict adherence to the manufacturer's Standard Operation Procedures (SOP) in carrying out the analysis.

Liver enzymes

Both AST and ALT were estimated using the method of Reitman and Frankel (1957). AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine (2,4-DNPH) read spectrophotometrically at 546 nm.

0.1 ml of serum was added to 0.5 ml of buffered AST substrate and incubated at 25°C for 20 min. 5.0 ml of sodium hydroxide (0.4 mol/l) was added at the end of 20 min, mixed and allowed for 5 min. The blank was set up in the same manner except for the addition of distilled water in the place of serum. The absorbance of the sample was read against the blank at 546 nm wavelength using a spectrophotometer.

ALT was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-DNPH read spectrophotometrically at 546 nm. The procedure is the same as AST except that 0.5 ml of buffered ALT substrate was used in the place of 0.5 ml of buffered AST substrate. AST and ALT activities were then obtained from the respective tables provided by the manufacturer in the SOP.

Alkaline phosphatase

The substrate, *p*-nitrophenyl phosphate (colourless), on hydrolysis catalyzed by ALP, produces phosphate and *p*-nitrophenol (yellow). The production of *p*-nitrophenol was monitored and measured spectrophotometrically at 405 nm (King and King, 1954).

To a tube containing 1.0 ml of buffered ALP substrate was added 0.02 ml of serum sample and mixed, the initial absorbance was spectrophotometrically read at 405 nm and absorbance reading repeated at 1, 2 and 3 min. ALP activity was calculated using the formula: u/l = 2760 x Δ 405/min.

Vitamin C (ascorbic acid)

Ascorbic acid is converted to dehydroascorbic acid by cupric ion

Table 2. Mean weight changes in Wistar rats treated with paracetamol.

Group	Initial weight (g)	Final weight (g)	Change in weight (g)
A	167±8.4	208 ±8.3	41± 6.2
B	184 ± 3.7	155 ± 4.4	-29 ± 3.1
C	171 ±5.8	177±5.6	6 ± 4.2
D	194 ±5.8	218 ±5.1	24 ± 4 .1

Table 3. Plasma levels of hepatic marker enzymes of various groups of paracetamol-treated Wistar rats treated with *A. indica* leaves aqueous extract, and the control group.

Group	AST (µ/l)	ALT (µ/l)	ALP (µ/l)
A	60.6 ± 3.98	62.8 ± 3.25	80.4 ± 2.33
B	106.2± 2.56	100 ± 4.1	86.8.2 ± 3.12
C	90.6 ± 3.44	90.6 ±3.93	84.2 ± 2.71
D	69.2 ± 2.48	65.6 ± 2.8	80.5 ± 3. 12

with 2,4-DNPH in the presence of thiourea as a mild reducing agent, sulphuric acid then converts DNPH to a red coloured compound which is read spectrophotometrically at 530 nm (Omaye et al., 1979).

0.5 ml each of serum and liver homogenate were added to different tubes and 1.5 ml of the standard add to another tube and to all the tubes were added 0.5 ml of DNPH reagent (92% DNPH in 0.9 N sulphuric acid, 4% thiourea and cupric sulphate solution), mixed and incubated at room temperature for 3 h. Thereafter, 2.5 ml of 8.5% sulphuric acid was added to each tube and the colour developed read spectrophotometrically at 530 nm after 30 min. The values were then calculated.

Vitamin E

Vitamin E reduces ferric to ferrous ions which then forms a red complex with x-x-dipyridyl. Vitamin E and carotenes were first extracted into xylene and extinction read at 460 nm to measure the carotenes. A correction is made for these after adding ferric chloride and reading at 520 nm (Quaife et al., 1949).

Statistical analysis

All values were expressed as mean ± SD and then subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago Illinois). Statistical significance was considered at $\rho< 0.05$.

RESULTS

Table 2 shows that there was a significant increase in the body weight of rats in groups A and D, and a decrease in group B ($\rho<0.05$). There was no significant change in the weights of Wistar rats in group C (p>0.05).

Table 3 shows that there was a significant increase in the plasma levels of the liver enzymes AST and ALT in group B as compared to those of group A (p>0.05). The plasma levels of AST and ALT in group C Wistar rats significantly increased when compared with rats in group A ($\rho<0.5$). However, these enzymes were significantly decreased when compared with rats in group B (p>0.05). There was no significant variation in plasma ALP level of group C from those of groups A and B rats (p>0.05).

Table 4 shows that the homogenate and plasma levels of vitamins C and E in group B were significantly decreased when compared with control group A Wistar rats (p>0.05). But there was no significant difference in these vitamins in groups C and D when compared with group A (p>0.05). The homogenate and plasma vitamins C and E levels in groups C and D were observed to be significantly increased when compared with group B (p<0.05). Analysis shows that the differences between the homogenate/plasma levels of both vitamins C and E in groups C and D were significant (p<0.05)

DISCUSSION

In this study, it appears that the overall metabolic effects of various treatments of the different groups of rats were summarized in their respective weight changes. The finding of this study shows a significant increase in the body weights of group A wistar rats, which is consistent with the normal physiological features as a result of normal metabolic processes in normally fed and conditioned animal. On the contrary, rats in group B show a significant decrease in the body weight, which may be attributed to the negative biochemical effect engendered by the paracetamol induced oxidative stress (Bhanwra et al., 2000). For groups C and D which were treated with paracetamol and varied doses of *A. indica* leaves aqueous extract, there was no significant weight increase in group C but significant increase in group D. This is reminiscent of the antioxidant effect of the extract

Table 4. Liver homogenate and plasma levels of vitamins C and E of different groups of paracetamol-treated Wistar rats treated with *A. Indica* leaves aqueous extract, and the control group.

Group	Vitamin C		Vitamin E	
	Homogenate (mg/dl)	Plasma (mg/dl)	Homogenate (mg/dl)	Plasma (mg/dl)
A	1.56 ± 0.26	1.64 ± 0.32	1.73 ± 0.36	2.42 ± 0.25
B	0.86 ± 0.3	0.91 ± 0.25	0.9 ± 0.28	1.1 ± 0.28
C	1.38 ± 0.32	1.38 ± 0.23	1.42 ± 0.30	1.8 ± 0.23
D	1.58 ± 0.25	1.56 ± 0.25	1.68 ± 0.32	2.3 ± 0.30

(Nwanjo and Orjiako, 2006), thus obliterating the effects of the oxidative stress, which could have been induced by paracetamol (Hazai et al., 2002).

The observed significant increase in the hepatic marker enzymes (AST and ALT) in paracetamol treated Wistar rats (group B) as compared to group A may implicate stress on the liver enzymes by paracetamol. This is further supported by the plasma AST/ALT ratio of less than one (AST/ALT <1) which may indicate ensuing acute or chronic liver injury (Essani et al., 1995). This could be due the fact that cellular enzymes extrude into the extracellular fluid, thus raising their concentrations in plasma (Adeyemi and Bukola, 2014) due to hepatocellular damage, hence the rise in their plasma levels. The significant decrease in AST and ALT observed in groups C and D when compared with group B may be attributed to the antioxidative or the hepatoprotective effect of leaves aqueous extract of *A. indica* which is similar to documented reports on the hepatoprotective activity of *A. indica* (Ha et al., 2010). The extract may have an effect on the Nrf2 regulation, thereby conferring the preservation of hepatocellular integrity against paracetamol-induced hepatotoxicity, which is in tandem with the work of Thomson (2013). The non-significant increase in plasma ALP in group B may mean that though paracetamol intoxication causes hepatocellular damage (Bhanwra et al., 2000), cholestasis may not be primarily involved.

Also, in this study, the result indicates that homogenate and plasma levels of vitamins C and E in group B are significantly decreased when compared with group A Wistar rats. But there was no significant difference in these vitamins in groups C and D when compared with group A. The depletion of vitamins C and E observed in the paracetamol intoxicated Wistar rats (group B) could be correlated with the excessive utilization of non-enzymic antioxidants in scavenging enormous free radicals produced and this corroborates with the widely studied role of antioxidants like vitamins C and E in xenobiotics-induced oxidative stress and hepatoprotection (Sharma et al., 2010). On treatment with both paracetamol and *A. indica* leaves aqueous extract, animals in groups C and D showed minimal or no change in levels of vitamins C and E when compared with the control group. The extract may have impacted

positively on the Nrf2 which in turn increased the production of antioxidant proteins which mopped up the oxidants, thereby conserving vitamins C and E. However, there is a significant increase when compared with group B. *A. indica* as an antioxidant prevents lipid peroxidation thereby reducing ROS. In the event of a reduced ROS generation, vitamins C and E are preserved, hence the increase in their values in groups C and D. The observed variations between the levels of biochemical parameters in groups C and D evaluated in this work with regards to varied doses of *A. indica* leaves aqueous extract used is shown to be significant. This could suggest that its effect in controlling oxidative stress and hepatocellular damage may be dose-dependent.

Conclusion

In conclusion, based on these findings, it could be inferred that *A. indica* leaves aqueous extract enhances vitamins C and E levels in paracetamol induced hepatocellular damage in Wistar rats. This could be due to its antioxidant activity and its effect on Nrf2 regulation. It, therefore, becomes pertinent to develop ways to modulate cell specific Nrf2 activity to facilitate the development of novel strategies for the treatment of oxidative stress-induced diseases.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

The antimalaria effect of *Momordica charantia* L. and *Mirabilis jalapa* leaf extracts using animal model

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Momordica charantia L. (Cucurbitaceae) and *Mirabilis jalapa L.* (Nyctaginaceae) are medicinal plants used extensively in almost all folklore remedies around the world to treat malaria. This experiment investigated the effects of *M. charantia* L. and *M. jalapa* L. on malaria in a 4-day suppressive test. Animals received 50, 100, or 200 mg/kg of methanolic extracts orally. *M. charantia* and *M. jalapa* methanolic extracts had intrinsic antimalarial properties that were dose-dependent. The result showed that *M. charantia* was effective in suppressing malaria at the highest dose tested (200 mg/kg) while *M. jalapa* gave the highest chemosuppression of parasitemia at the lowest tested dose of 50 mg/kg body weight of mice. The result also showed that the standard reference drug, Chloroquine, had its highest chemosuppression of parasitemia (100%) at 20 mg/kg when administered orally. This research affirms the uses of these plants for the treatment of malaria.

Key words: Antimalarial, *Plasmodium berghei, M. charantia, M. jalapa.*

INTRODUCTION

Malaria is still one of the most significant diseases in the world. The World Health Organization (WHO) reported that half of the world's population is prone to malaria in which 1-2 million deaths occur annually (WHO, 2012; Vogel, 2010).

Plasmodium falciparum, among the protozoan species of the genus *Plasmodium,* causes most of the severe cases of this ailment (Nogueira and Lopes, 2011). Many drugs have been used to treat malaria e.g. quinine, chloroquine, mefloquine, artemisinin amongst others, but

the parasite has developed resistance against a lot of these treatment regimens (White, 2004).

In the search for new therapeutic substances, a great number of researchers have resorted to plant sources (Chin et al., 2006; Fabricant and Farnsworth, 2001; Addae-Mensah et al., 2011). This is due to the fact that many of these plants are used in African traditional medicine (ATM) (Ginsburg and Deharo, 2011) and drugs from natural products have been summon for use as origin of development of new antimalarials (Guantai and

Chibale, 2011; Cruz et al., 2013; Wells, 2011; Anthony et al., 2012) so as to eradicate drug resistance problems (Taylor, 2002).

Momordica charantia (Family: Cucurbitaceae, Plate 1) is widely called bitter melon, bitter gourd, Balsam pear, Karela and Pare. It develops naturally in tropical areas of the Amazon, East Africa, Asia, India, South America, and the caribbean. The plant is perennial, herbaceous and tendril climber which grows to six meters or longer. It has a lengthen fruit that looks like a warty gourd or cucumber. The unripe fruit is either white or green in colour and has a bitter taste that becomes more noticeable as the fruit ripens. The Latin name *Momordica* means "to bite" (describing the uneven edges of the leaf, as if they had been bitten) (Bakare et al., 2010). The leaves are simple, alternate (4-12 cm across) and palmately veined having ununited 3-7 deep lobes. *M. charantia* is a strong nutrient- based herb which consists of a composite collection of phytochemicals such as bioactive compounds, vitamins, minerals and antioxidants that contribute to its extraordinary ability in treating a whole lot of sicknesses. Bitter melon has separate yellow male and female flowers. The leaves and fruits of *M. charantia* are rich in vitamin A, vitamin B, vitamin C, vitamin E, iron, calcium, phosphorus and beta carotene. They are also rich in dietary fibers. The values of the calories present in leaf, fruit and seed were 213.26, 241.66 and 176.61 Kcal/100 g respectively (Snee et al., 2011). The activity of bitter melon has been ascribed to the level of antioxidant in it (Kirtikar and Basu, 2001).

A steroid saponin called charantin and a polypeptide named gurmarin, which is similar to insulin in composition were isolated from the fruits and leaves of *M. charantia*. These bioactive constituents were reported to be responsible for its hypoglycemic activity (Raman and Lau, 1996). *M. charantia* has some interesting biological and pharmacological activities. Previous investigations have shown that aqueous extracts of the leaf and fruit of *M. charantia* exhibited high antioxidant activity (Kubola and Siriamornpun, 2008). The role of free radicals and active oxygen in treating chronic diseases including cancer, aging and atherosclerosis has been recognized (Mathew and Abraham, 2006). Therefore, much attention has been focused on the use of antioxidants in protecting against the threat of damage of free radicals. It is a wonderful herbal medicine for human health. *M. charantia* is used in folkloric medicine to treat diabetes, HIV, coughs, skin diseases, sterility in women, parasiticide, antipyretic and as purgative among others.

M. jalapa (Plate 2) belongs to the family Nyctaginaceae which is popularly known as beauty of the night, four o'clock, or marvel of Peru. It is an herbaceous climber growing up to 2 m high. It has opposite leaves, very big impressive flowers, curvaceous, obovoid fruits and conspicuous tuberous roots. *Mirabilis* in Latin means 'wonderful' and Jalapa is a popular name in Central and North America. The precise source of *M. jalapa* is unknown, but it is believed to originate from parts of tropical America (Yi-Fen et al., 2002). It is approximately 0.9 m high. It is mostly grown among the species of *Mirabilis* and has diverse of colours. Each flower is spattered with different colours and the designs are known as sectors (whole sections of flower), flakes (stripes of varying length), and spots. A flower can be plain yellow, pink or white, or mixture of sectors, flakes and spots (Miko, 2008). *M. jalapa* is also known for its colour-changing attribute.

For instance, yellow variety flower changes to dark pink colour gradually as it matures, so also is white flowers which change to light violet. The flowers normally unfold from late afternoon onwards, which led to its name 'the four o'clock plant'. The flowers produce long lasting sweet-smell all through the night, and fold up in the morning. The flower of *M. jalapa* developed from the pigmented modification of the calyx and not from the petals. It produces flowers from July to October, and the seeds ripen from August to October. The fruits with single seed are spherical, wrinkled and black when matured (Wang Yi-Fen et al., 2002). Several components such as β-sitosterol, stigmasterol, ursolic acid, oleanolic acid, brassicasterol, and *Mirabilis* antiviral protein, rotenoids (mirabijalone A-D, boeravinones C and F) were isolated from the aerial parts and roots of *M. jalapa* (Siddiqui et al., 1990, Siddqui et al., 1994; Yi-Fen et al., 2002). Aoki et al. (2008) and Oskay et al. (2007) reported that *M. jalapa* had numerous biological activities such as antispasmodic, antibacterial, antiviral, antifungal and protein synthesis inhibition. *M. jalapa* is used in herbal medicine for the treatment of diarrhea, dysentery, conjunctivitis, edema, inflammation, swellings, muscular pain and malarial (Daniel, 2006).

In this work, we evaluated the antimalarial activities of *M. charantia* and *M. jalapa* using animal model with the view to justify the ethnomedicinal claim of the indigenous usage of the species in the cure of malaria.

MATERIALS AND METHODS

Plant specimens

The plant specimens used for this study were fresh leaves of *M. charantia* and *M. jalapa*. They were collected at the botanical garden of Adekunle Ajasin University, Akungba Akoko and authenticated in Forestry Research Institute of Nigeria (FRIN). The voucher sample was deposited at FHI (Forest Herbarium, Ibadan) with herbarium numbers FHI 110131 and 110132 respectively. *M. charantia* (179 g) and 160 g of *M. jalapa* leaves were air-dried, powdered and macerated with 70% methanol for five days. The filtrates were concentrated to dryness *in vacuo* and weighed (89.88 and 91.95 g respectively). Dilutions of dried extracts were prepared to give appropriate concentrations used for the assay.

Preliminary phytochemical analysis

Phytochemical screening of the plants was carried out using standard procedures to test for alkaloids, saponin, cardiac

Plate 1. *Momordica charantia* L.

Plate 2. *Mirabilis jalapa* L.

glycosides, steriods, flavonoids and tannins (Sofowora, 1993; Trease and Evans, 1986).

Alkaloids

1 g of powdered sample was stirred in 10 ml of 10% (v/v) HCL on a steam bath followed by filtration. The filtrate (1 ml) was mixed with a few drops of Meyer's reagent. To another 1 ml of the filter was added few drops of Wagner's reagent and a few drops of Drangendorff reagent was added to another 1 ml of the filtrate. The mixtures were observed for turbidity or formation of precipitate.

Saponins

1 g of powdered sample was boiled with 10 ml of distilled water for 10 min. The sample was filtered while hot, cooled and the following tests were performed:

1. Frothing test: 2.5 ml of the filtrate was diluted to 10ml with distilled water and shaken vigorously for 20 min. The formation of persistent foams was taken as evidence for the presence of saponins.

2. Emulsifying property: 2 drops of olive oil were added to 2.5 ml of

the filtrate and shaken vigorously for 30 min. Observation was made for the formation of stable emulsion.

Tannins

1 g of powdered sample was boiled in 10ml of distilled water, filtered whilst hot and cooled. The filtrate was adjusted to 10 ml with distilled water. Then a few drops of 1% ferric chloride regent were added to 1 ml of the filtrate. The mixture was observed for the formation of blue, blue black, green-black colouration or precipitate.

Flavonoids

1 g of powdered sample was boiled with 10 ml of ethanol.

1. To 5 ml of the extract was added 2 drops of ferric chloride. A dusty green colour was considered positive.
2. To 5 ml of the extract, a small quantity of dilute NaOH was added and drops of Conc. HCL were run down the side of the tube. A reddish colouration indicated the presence of flavonoids.

Cardiac glycosides

1 g of the sample was extracted with 10ml of 80% ethanol for five minutes on a water bath. The extract was filtered and diluted with equal volume of distilled water. A few drops of lead acetate solution were added, shook and filtered after standing for a few minutes. The filtrate was then extracted with aliquots of chloroform; the extract was divided into two portions in evaporating dish and evaporated to dryness on a steam bath.

Keller killiani test

One portion from above was dissolved in 2 ml of glacial acetic acid containing one drop of $FeCl_3$ solution in a clean test tube. 2 ml of concentration sulphuric acid was then poured down the side of the tube so as to form a layer below the acetic acid. The formation of a purple or reddish-brown or brown ring at the interface and a green colour in the acetic layer was taken for positive result (Sofowora, 1993).

Kedde test

The second potion was mixed with 1ml of 2% 3, 5-dinitrobenzoic acid in ethanol. The solution was made alkaline with 5% NaOH after mixing. The formation of a transient purple, which turned brown on standing, was considered positive.

Steroids

1 g methanolic extract was dissolved in 1 ml acetic anhydride and then 1 ml of dichloromethane. The solution was transferred into a dry test tube and by the means of pipette 2 ml of concentrated sulphuric acid was added at the bottom of the test tube. At the contact zone of the two liquids, a brownish-red ring was formed; the supernatant layer became greenish denoting presence of sterols and triterpenes.

Experimental animals

Adult Swiss albino mice weighing between 20 and 40 g of both sexes were used for the estimation of antimalarial properties of the plant extracts. They were obtained from the animal house, Department of Physiology, University of Ibadan. All experimental protocols were in accordance with internationally accepted principles for laboratory animal use and care as found in the US guidelines (Makinde et al., 1988). The animals were caged under standard conditions and fed with a stock diet and water *ad labitum.*

Parasites

The antimalarial activities of the methanol extract of *M. charantia* and *M. jalapa* leaves were evaluated with chloroquine-susceptible strain of *Plasmodium berghei* (NK 65). Parasite was acquired from the Malaria Research Laboratories, Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan.

Assessment of early malarial infection (4-day suppressive test)

The antimalarial activities of *M. charantia* and *M. jalapa* leaves were investigated using a 4-day suppressive test in *P. berghei*-infected mouse model (Peters and Robinson, 1992; Tona et al., 2001).

Twenty adult Swiss albino mice were grouped into five of four each and the mice that donated the parasite were infected with 200 µl of *P. berghei* inoculum. The blood of each donor mouse that had been infested with parasite was collected from the tail vein and thinned with 0.9% sodium chloride. Normal saline suspension of 1×10^7 parasitized erythrocytes (0.2 ml) was introduced into the mice by intra-peritoneal (i. p.) injection (Day 0). Four hours later, the first three groups were treated with 50, 100 and 200 mg/kg/day doses of the extracts for four successive days, while the fourth and fifth groups were treated with 20 mg/kg/day of chloroquine diphosphate (positive control) and 5 ml of normal saline (negative control) respectively for four sequential days. On the fourth day, thin blood films were prepared from blood collected from the tails of all mice. The films were air-dried, fixed in methanol for 30 s, and stained with 10% giemsa for 20 min on the previously washed slides. Parasitaemia of each mouse was counted under microscope and the percentage of suppression of parasitaemia for each dose was calculated:

$$\% \text{ Suppression} = \frac{\text{Parasitaemia of negative control} - \text{Parasitaemia of test drug}}{\text{Parasitaemia of negative control}} \times 100$$

The reduction in the percentage of parasite denotes the antimalarial activities of the extracts. (Philipson and Wright, 1991). Results were represented as mean values. Comparison of difference in quantitative variables between more than two and two groups was performed using Analysis of Variance (ANOVA) tests (SPSS version 16.0, SPSS Inc., CO, USA). Statistical significance level was set at $P < 0.05$ for all tests.

RESULTS AND DISCUSSION

The percentage yields of the crude extracts were 57.50% w/w for *M. jalapa* leaves and 50.21% w/w for *M. charantia* leaves. Results of the phytochemical screening (Table 1) showed the presence of some bioactive components in the leaves. They contain alkaloids, saponins, tannins, flavonoids, cardiac glycosides and steroids. It was noted that results of the screening showed abundant presence

Table 1. Qualitative phytochemical analysis of *M. omordica charantia* and *M. jalapa* leaves.

Test	Observation	*M. charantia*	*M. jalapa*
Alkaloids			
Dragendorff's reagents	Reddish brown precipitate	+++	+++
Mayer's reagents	Pale cream precipitate	+++	+++
Wagner's reagents	Reddish brown precipate	+++	+++
Saponins			
Frothing Test	Persistent frothing	++	+++
Emulsification	Oily layer emulsified	++	+++
TANNINS	Greyish green colouration	+++	+++
Flavonoids			
Ferric Chloride solution	Dusted green colour	+++	+++
Reaction with Sodium Hydroxide	Reddish-brown colouration	+++	+++
Cardiac-glycosides			
Keller-Kiliani test	Brown ring formed at interphase	++	++
Kedde test	Brown purple precipitate	++	++
Steriods			
Acetic anhydride and chloroform	Solution turns brown	++	++
Concentrated sulphuric acid	Brown-red ring formed at interphase and supernantant layer turns green	++	++

+++. Highly positive; ++, positive.

Table 2. Antimalarial activity of methanolic leaves extracts of *M. charantia* and *M.irabilis jalapa* in animal model.

Test drug dose (mg/kg body weight/day)	*M. charantia* methanolic extract		*M. jalapa* methanolic extract	
	Parasitaemia (%)	Chemosuppression (%)	Parasitaemia (%)	Chemosuppression (%)
50	$2.40^a \pm 3.09$	60.39	$1.05^a \pm 1.84$	82.67
100	$0.82^a \pm 1.45$	86.46	$2.86^a \pm 2.90$	52.80
200	$0.00^a \pm 0.00$	100	$5.77^b \pm 1.89$	4.78
Chloroquine	$0.00^a \pm 0.00$	100	$0.00^a \pm 0.00$	100
Normal saline	$6.06^b \pm 0.20$	-	6.06 ± 0.20	-

Value is represented as mean ± SD, n=4. Different alphabets in the same column denote significantly different values ($p<0.05$) as separated by analysis of variance.

of alkaloids, saponins, tannins and flavonoids in *M. jalapa* leaves and alkaloids, tannins, flavonoids and steroids in *M. charantia* leaves.

Phytochemicals such as terpenoids (e.g. Artemisinin) are involved in the antiprotozal and antiplasmodial potential of diverse plants (Francois et al., 1996; Ghoshal et al., 1996; Asase et al., 2010; Tasdemir et al., 2006). Flavonoids exhibit substantial antiparasitic potentials against different strains of malaria, trypanosome and leishmania (Waako et al., 2007). There were records that alkaloids derived from plants have a lot of contributions to the development of anti-malarial drugs (Schwikkard and Van Heerden, 2002; Bero et al., 2009). *M. charantia* and *M. jalapa* plants are popularly used in herbal medicine to

cure malaria. The results of this experiment revealed antimalarial activities (Table 2) for the *M. charantia* and *M. jalapa* in the 4-day suppressive antimalarial test in mice infected with *Plasmodium berghei* (NK 65). The results showed high antimalarial potency when compared with the results of the standard reference drug (chloroquine) which gave 100% at the dose of 20 mg/kg. The bioassay carried out on the plants gave varying results. The mean percentage parasitaemia of *M. charantia* was 2.40 ± 3.09, 0.82 ± 1.45 and 0.00 ± 0.00% at 50, 100 and 200 mg/kg respectively, while positive and negative controls gave 0.00 ± 0.00 and 6.06 ± 0.20% respectively; the mean percentage parasitaemia of *M. jalapa* was, 1.05 ± 1.84, 2.86 ± 2.90 and 5.77 ± 1.89% for
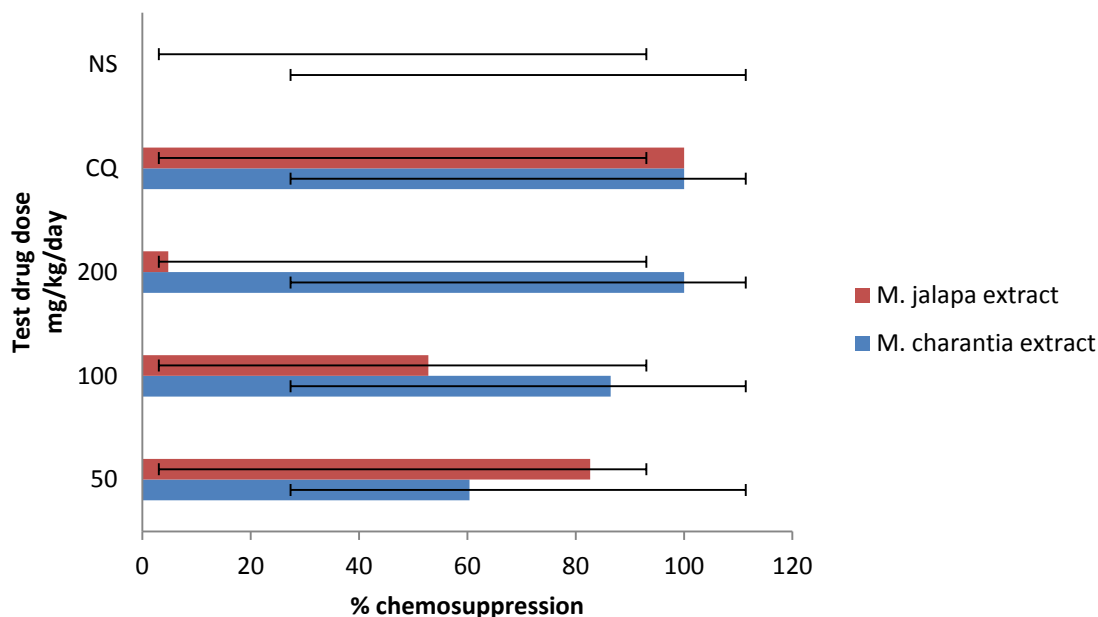
Figure 1. Antimalarial activity bar chart showing % chemosuppression of *Momordica charantia* and *Mirabilis jalapa* methanolic leaves extract.

50, 100 and 200 mg/kg respectively, positive (chloroquine) and negative (normal saline) controls also gave 0.00 ± 0.00 and 6.06 ± 0.20%, respectively. Figure 1 showed the mean percentage chemosuppression for *M. charantia* and *M. jalapa* leaves. The values for *M. charantia* were 60.39, 86.46 and 100% at 50, 100 and 200 mg/kg respectively, while positive and negative controls gave 100 and 0%, respectively. The mean percentage chemosuppression of *M. jalapa* were 82.67, 52.80 and 4.78% for 50, 100 and 200 mg/kg respectively, positive and negative controls gave 100 and 0% respectively. *M. charantia* had its lowest mean percentage parasitaemia at the dose of 200 mg/kg (0.00 ± 0.00%) while *M. jalapa* had its lowest mean percentage parasitaemia at the dose of 50 mg/kg (1.05 ± 1.84%). *M. charantia* leaves extract induced the highest chemosuppression of parasitaemia (100%) at dose of 200 mg/kg compared to chloroquine (20 mg/kg), positive control group which had a chemosuppression of 100% while *M. jalapa* leaves had its highest activity of 82.67% at 50 mg/kg. The standard drug, chloroquine however gave 100% chemosuppression at 20 mg/kg. *M. charantia* leaves (100%) showed a significantly ($p<0.05$) higher percentage chemosuppression of parasitaemia than *M. jalapa* leaves (82.67%). The values were observed to increase as extract concentration increased in *M. charantia* leaves and decreased as extract concentration increased in *M. jalapa* leaves that is, the activity is dose dependent.

The activities might be attributed to the presence of one of the phytochemicals identified to be present; or even a combined action of more than one of the metabolites.

However, the active compound(s) known to give this observed activity need to be identified. In this regard, efforts are presently directed towards biologically guided fractionation of these plants so as to isolate, identify and characterize the active metabolite(s) and also investigate on its cytotoxic activity.

Conclusion

This current research revealed the potency of *Momordica charantia* and *Mirabilis jalapa* methanolic leaves extracts against malarial *in vivo.* Therefore, the use of these plants traditionally for malarial is justified.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Farmer's knowledge level and training needs toward the production and conservation of medicinal herbal plants in Jordan

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The main purpose of the study is to determine farmer's awareness and training needs towards the production of Medicinal and Herbal (M/H) plants in Jordan. A survey questionnaire described the personal characteristics of M/H farmers with sample size population of 209. Results showed the majority of farmers grow thyme, sage, and mint which is a popular M/H plant grown in Jordan. A majority of farmers, 71%, feel that there is a high need for a proper legislation for conserving M/H plants in Jordan. The majority of illiterate farmers 0.89%, indicated a very high need for awareness programs about conservation and production of M/H plants. On the other hand, the majority of farmers with university degree believe that M/H plants should be conserved in their natural habitat and should be protected by legislations. Female farmers, 50%, displayed a sense of awareness than the males as regards various methods of drying. Also, the females, 42%, do not know much about implementing the irrigation system in their fields and 43% are not aware of the maintenance of the irrigation system. The study indicates the priorities of the following needed training courses: Marketing, Pest and disease management, Harvesting and post-harvesting techniques, Irrigation design and maintenance, Fertilizer application, Soil sterilization, Planting methods, Weed control, and Soil preparation. This study is considered as the first in Jordan that describes M/H plants farmers and their priority training needs.

Key words: Extension, farmers awareness, Jordan, Medicinal and herbal (M/H) plants, production and conservation, training needs.

INTRODUCTION

Herbal medicine refers to the use of herbs for their medicinal value. A herb is a plant or a plant part valued for its medicinal, aromatic or savory qualities. Usually, herbalists use leaves, flowers, stem, berries, seeds, whole plant and roots of plants to prevent, relieve, and treat illness. Historically, herbal medicine is the oldest form of health care that had been used by all cultures. Throughout the middle ages, homegrown botanic are the

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only medicines readily available and for centuries, no self-respecting household would be without a carefully tended and extensively used herb garden. In most parts, herbal healing was passed from generation to generation by means of children being taught by their mothers (Shizha and Charema, 2011). People through their exploration, conquest and most importantly, the desire to aid the sick, ancient civilizations tended to borrow and adopt the skills, knowledge of medicine and healing of various cultures to their own (Sumner, 2000).

Medicinal plants are worldwide and are heavily used in traditional medicine (Obeidat, 2011). World Health Organization (WHO) estimations (80%) of the world's population relies on medicinal herbal plants for their primary health care needs (Farnsworth et al. 1985). This causes increased global interest in plant biodiversity as a means of providing more stable and secure sources of food and non-food product to meet future threats such as rising food costs, population increases, climate change, rapidly changing pest and disease threats, withdrawal of pesticides due to regulation (Craker and Simon, 1986). Earlier, medicinal plants have been used with multi-functional properties including nutraceutical food components, medical usage, and functional food. Health effects of medicinal plants have been linked with its consumption of active compounds like volatile oils, phenolic and peptide compounds that lead to decrease risk of the antiviral activity, cardiovascular disease, antifungal activity, antibacterial activity, anti-inflammatory and laxative effect (Shahidi and Naczk, 2004; Khalil et al., 2012).

The bulk of the raw materials used in preparing drugs are mostly collected from wild nature, this heavy collection causes a major problem for medicinal herbal plants. Natives to developing countries often export medicinal herbal plants to developed countries as raw materials where they are screened, analyzed and used in drug preparations to be returned as high priced medicines (Shizha and Charema, 2011). The heavy reliance on plant medicine in developing countries is attributed to their relative accessibility, low prices, local availability, and acceptance in local communities and the low number of dispensaries and doctors needed for healthcare especially in rural areas (Conserve Africa, 2005).

Generally, there are over 750000 M/H plants in the world. Medicinal plants fall into two categories, wild grown and farm grown. Wild grown herb grows naturally as a result of natural selection without human intervention, and the process of gathering herbs from their natural habitat is called wildcrafting. On the other hand, medicinal and herbal plants (M/H) grown on the farm are nurtured through cultivation and propagation processes. However, for producing high-quality products, herb farmers require a great deal of specialized knowledge. In developing countries, herbal medicine is a major component in the indigenous peoples' traditional medicine. Moreover,

economic analyses have shown that medicinal plants have considerably contributed to the economic welfare of people by providing and generating reasonable income. They also contribute to household self-sufficient food security through the accumulation of savings and minimization of risks. WHO notes that of the 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures (Drew and Myers, 1997).

Conservation of medicinal plants, especially endangered species depends largely on the conservation of the ecosystem in which they grow. The availability of medicinal plants is of vital importance for herb collectors and also for the future rapid growing industry worldwide; therefore, conservation of M/H plant is an important issue (World Bank, 2003). Horticultural research on medicinal plants has focused on developing the capacity for optimal growth in cultivation. This has been especially pertinent as many medicinal plants are still harvested in the wild, and conditions for growth and cultivation have not been optimized (Briskin, 2000). The recent call for "back to nature" has affected all public sectors, probably because some synthetic drugs failed to prove effectiveness with serious effect (Abu Irmaileh and Afifi, 2003). The farming system would give commercial cultivation such as *Echinacea purpurea* in Taiwan. Transplanting *E. purpurea* seedlings in the autumn season and harvesting the aerial parts at the beginning of winter season, and then harvesting the rhizome-regenerated plants again in the following summer season are technically and commercially feasible (Chen et al., 2008).

The most commonly adopted approach toward conserving threatened species of M/H is to create awareness, develop cultivation, propagation techniques at the community level and provide local farmers with greater access to reliable and profitable markets. Furthermore, technical advice dedicated to promoting M/H plants based on recommendations arose from agricultural research (Faure et al., 2013). Therefore, herbs collectors need to be aware and trained on when and how to harvest. From the above facts, M/H plants should be conserved in a way that maintains and sustain their benefits, especially by conserving the natural habitat of these plants. Although Jordan is relatively a small country, it is characterized by a great variation of wild plants (Al-Quran, 2011). Medicinal herbal plants in Jordan are distributed all over the country from the eastern desert to the western highlands and from the semiarid north to the extremely arid south. The flora of Jordan is rich in medicinal and aromatic plants, as well as herbs and species, mainly those belong to the Umbelliferae, Labiatae, and Compositae families. Oran et al. (1994) found a total of 485 species which belong to 330 genera, and 99 families are herbs, shrubs, or trees and they comprise 25% of the total flora in Jordan. Many of the medicinal and herbal plants in Jordan are

Figure 1. The map of Jordan (study area).

endangered, while some are threatened with extinction. The continuous and accelerating over-exploitation of these plants in their natural habitats, combined with the increasing demands on them, have led to the destruction of natural stocks in the wild. Therefore, evaluating the level of farmer knowledge and training needs for conserving and producing M/H plants is of crucial importance.

The main purpose of this study is to inform and train the farmer as regard the production of medicinal herbs for the sustainable use and trade in the region. The specific objectives were to describe M/H farmers on the following characteristics: gender, age, educational level, farm size, experience, work status, marital status, family size, % income from cultivation of M/H plants, determining farmer's awareness towards the production of M/H, the relationship between farmer's awareness and certain personal characteristics of farmers, and determining the training needs of farmers in the production of the M/H plants.

MATERIALS AND METHODS

Jordan (31°00′N 36°00′E) is classified as being located in semi-arid to the arid region (Figure 1) and suffers from inadequate availability of water resources that are also unevenly distributed throughout the kingdom area (Dahamsheh and Aksoy, 2007; MWI, 2014). Jordan occupies an area of 89,297 km^2 (MWI, 2014) and has a precipitation rate that varies from a minimum of 50 mm in the desert to 600 mm in northwest highlands (Tabieh et al., 2010). More than 90 % of Jordan area receives a total annual precipitation quantities below 200 mm (MWI, 2014) with an evaporation rate of 93.9 % (Hadadin et al., 2010).

The descriptive correlational research method was used in conducting this study and was used to come up with precise recommendations that enhanced and increased the production of M/H plants. The research instrument is a survey questionnaire consisting of the personal characteristics of farmers which include: age, gender, educational level, family size, farm size, work status, family size, social status and income percentage from M/H plants and the farmers experience. In order to determine the general knowledge of farmers in various types of M/H plants, twenty-seven types of M/H plants were recommended and focused on by (M/H) project and listed in the questionnaire for farmers to identify their

Table 1. Personal characteristics of medicinal herbal plant (M/H) farmers.

Gender	Percentage	Marital status	Percentage	Age of farmers	Percentage	Educational level	Percentage	Farm size (ha)	Percentage	Experience /year	Percentage	Work status	Percentage
Male	66	Married	86	<30	11	unschooled	9	<1	32	<5	24	Full time farmer	32
Female	34	Single	12	31-40	33	Primary	11	1-5	23	6-10	24	Part time	21
-	-	other	2	41-50	27	Secondary	20	6-10	11	11-15	20	Owner	32
-	-	-	-	51-60	18	High school	24	11-15	7	16-20	9	Rental	9
-	-	-	-	>60	12	University	36	>15	27	>20	23	Owner +rental	6

types. Moreover, this part of the instrument designed to ask about other types of M/H plants that farmers can identify was followed by questions regarding the types of M/H plants that farmer grow. In addition, the questionnaire was designed to determine farmer's knowledge and awareness level toward the productions of medicinal herbal plants in Jordan, and it comprised of 64 Likert-type scale questions. These questions are related to economic, nutritional, medicinal and ecological values, methods of using Likert-type M/H plants, extension, and cultural practices. Furthermore, these 64 questions were used to determine the relationship between the knowledge level of the management of medicinal plants and certain personal characteristics of farmers such as age, gender, educational level, family size, farm size, work status and percentage of income. Also, the questionnaire dealt with the rank order, the source of knowledge and information of farmers. The questionnaire likewise included 14 questions about the communications of farmers with agricultural extension services in their areas. Finally, the questionnaire consisted of nine categories of identified training needs. Respondents can determine and prioritize their training needs. This part of the questionnaire also includes questions to determine and prioritize the obstacles of M/H production from the farmers point of view.

Validity and reliability of the research Instrument were established by a panel of experts and a field test. The estimated target population of the study is around 1000 farmers according to the Ministry of Agriculture / Agriculture Extension Department (2007). The sample size was n=209 farmer which represents about 20% of the target population. Simple random and cluster random was used in the sampling procedure.

The main method of data collection was personal interview questionnaire. Along with the main investigator of

this research, a team of data collectors was trained to collect the data during the period of three months. The descriptive statistics and correlation were used in analyzing the data. The data were analyzed using the Statistical Package for the Social Sciences (SPSS 11.5/ Windows) computer program.

RESULTS AND DISCUSSION

The trade of M/H plants resulted in debatable issues that includes endangering the survival of the species, erodes genetic diversity of the species, threatens their survival or functional integrity, and non-sustainability issues which in its most basic form means that the species is extracted from a particular site at a rate greater than that at which it is being replaced (Hamilton, 1992). Therefore, to ensure long-term gains to the indigenous communities and prevent over-harvesting, research findings and analysis suggested many recommendations and strategies such as conservation and generating awareness about the importance of M/H plants and its sustainable use.

The cultivation of M/H plants in Jordan is a recent practice and as a result, very few species are under cultivation. The experience of farmers in cultivation is still undeveloped and is growing gradually. At the beginning, cultivation was limited to few crops under irrigation and then extended to

the rain fed areas. The majority of farmers who cultivate M/H plants are concentrated in Irbid, Amman and Karak counties in Jordan. This is because land receives high rainfall, have irrigation sources and have the active farming communities. Moreover, non-governmental organizations carry out activities in these areas that encourage farmers to cultivate M/H plants and provide to them the proper technologies (Hadad and Turk, 2002).

The data presented in Table 1 shows that males are the majority of the sample population (66%) and about one-third of the samples are females. The age of the farmers were distributed as follows: one-third of the farmers, 33%, were between 31 to 40 years old; 27% were between 41-50 years; 11% werre less than 30 years old; 12% were above 60 years old. Regarding the educational level of farmers, more than one-third of the sample population was highly educated with university degrees, more than half of the sample population were with some form of education ranging from Primary to High school, and about 9% were unschooled. As for farm size, the study showed that one-third of farmers cultivate less than 0.1 ha. On the other hand, 27% of farmers cultivates more than 1.5 ha. Moreover, farmers who participated in the study varied in their experiences. Almost a quarter of the sample population have experience less than 5 years,

Table 2. General awareness.

Awareness	Percentage
High awareness	60
Medium awareness	17
Low awareness	23

24%, and approximately the same % of farmers have over 20 years of experience, 23%. Also, the study showed that one-third, 32%, of farmers are full-time farmers and own their lands while 9% of farmers rent their land. In addition, the study showed that majority, 86%, of the farmers are married and 12% are singles. Furthermore, the study showed that the mean average family size was 7 household members. Asking farmers about their percent of income generated from the cultivation of M/H plants, the study showed that the average generated income was 12% of the net total of production.

Haddad and Turk (2002) opined that the majority of Jordanian farmers own their land and around 50% of them have an area less than 0.2 ha. The areas devoted to M/H plants from farm area ranging from 0.1 ha to more than 5 ha with 53%, cultivated an area of 0.6 to 2 ha; 32%, less than 0.5 ha and only 6% cultivated more than 5 ha. Medicinal and herbal crops currently grown in Jordan under irrigation are Oregano, mint, and sage. Oregano and mint are grown mainly under plastic houses, but sage is grown in the open field. Crops grown under rainfed are cumin, black cumin, anise, and fenugreek. All available medicinal herbal plants species of Jordanian origin are consumed directly by inhabitants and collected from the wild with insignificant cultivation for few of them except for oregano, sage, anise and chamomile. For determining farmer's awareness towards the production of M/H plants as shown in Table 2, the study indicates that 60% of farmers are highly aware of the conservation and production aspects of M/H plants. At the other end, the study showed that only 23% of farmers have little awareness, and about 17% are averagely aware. However; regarding the general awareness and identification of M/H plant types, the study showed that 98% of farmers easily identify thyme, sage, and mint. The findings also indicated that the popular M/H plant grown in Jordan by majority of farmers are thyme, sage and mint.

To determine the relationship between farmers' awareness and certain personal characteristics of farmers as shown in Table 3, the following results show only the significant relationship between farmers' awareness and certain personal characteristics of farmers by using the Chi-square. The study showed that the majority of females, 64%, are high aware about conserving wild M/H plants that can help in biodiversity. Moreover, 50% females appeared to be more aware than males as

regards various methods of drying. On the other hand, 42% females displayed little knowledge on to implement the irrigation system in their fields and 43% are not aware of the maintenance of the irrigation system.

Furthermore, the association between farmers awareness and the age characteristics in the study showed that the majority of farmers, 60%, who are above 60 years are against harvesting M/H plant by pulling. 71% of the same age category of farmers feels that there is a high need for a proper legislation for conserving M/H plants in Jordan. According to the relationship between farmers' awareness and their educational level, the majority of illiterate farmers, 89%, displayed a very high need for awareness programs about conservation and production of M/H plants. The majority of farmers with university degrees believe that M/H plants should be conserved in their natural habitat.

The results of the relationship between farmer's awareness and farm size showed that 72% of farmers who own less than 0.1ha are very highly aware about the best way of using M/H plants is soaking. The same category of farmers are also highly aware in the knowledge of different methods of drying M/H plants, and on how to implement and maintain the irrigation system in their fields. Also, the same categories of farmers believe that most of the M/H plants should be irrigated by a drip irrigation system.

Farmers owning more than 1.5 ha, 53%, showed that they know different types of chemical fertilizers that can be applied to M/H plants and 52% are of the knowledge that majority of M/H plants require supplement irrigation 2 to 3 times per week. On the other hand, the majority of farmers in the category of less than 0.1 ha indicated that extension officers seldom advise them and this same category of farmers, 42%, have little knowledge of the the various types of fertilizers that can be added to the M/H plants.

The results of the relationship between farmer's awareness and farmers' experience indicated that farmers having experience more than 20 years, 74% showed very high awareness about quality control related to M/H plants. The same category of farmers, 70% believe that wild M/H plants should be protected by legislations and 71% believe that pulling the plants from the wild is not the proper way of harvesting.

Determining the training needs of farmers in the production of the M/H plants as shown in Table 4. The study showed that the first training courses required by farmers, 72%, is marketing (local and international). The second training course identified is training courses related to the identification of pests and diseases, Integrated Pest Management (IPM) control, methods of using pesticides and safety period, 65%. The third highest training needs, 53%, is training courses in harvesting (harvesting time, the method of harvesting, grading, packaging, cooling and storing of fresh and dried M/H plants). The fourth training need is irrigation design

Table 3. Determining the relationship (association) between level of knowledge on the management of medicinal plants and certain personal characteristics of farmers.

Varaible	Question	% of very high	% of high	% of low	% of very low
	I know nutritional benefits of M/H	M (23)	M (23)	M (6)	F (4)
	The best way of using M/H plants is boiling	M (10)	M (14)	M (20)	F (16)
	The best way of using M/H plants is soaking	M (31)	M (14)	M (4)	M (5)
	I know the way of putting irrigation system	M (39)	M (17)	F (14.1)	F (42)
	I know the maintenance of irrigation system	M (38)	M (18)	M (17)	F (43)
	I know types of chemical fertilizers added to M/H plants	M (28)	M (25)	F (17)	F (32)
Gender	I believe in add ding chemical fertilizers after harvesting	M (32)	M (34)	M (9)	F (31)
	I know most diseases that affect M/H plants	M (16)	M (28)	F (28)	F (28)
	Aphids considered as main insect that attack M/H plants	F (47)	F (26)	M (13)	M (0)
	I know different method of drying M/H plants	F (50)	F (33)	M (10)	M (13)
	I think it's possible to store fresh M/H plants for long period	F (32)	M (25)	M (11)	F (29)
	I participate in seminars and training courses specialized in M/H plants	F (33)	M (17)	M (17)	M (39)
	I believe that conserving wild M/H plants help in biodiversity	F (64)	F (19)	F (7)	F (7)
Age	I believe that the best way of harvesting wild M/H plants is pulling it out	41-50 (19)	41-50 (11)	<30 (35)	>60 (60)
	I believe that protecting M/H plants needs a law	>60 (71)	<30 (35)	31-40; 51-60; 41-50; (7)	31-40 (37)
	The best way of using M/H plants is boiling	Ill (28)	Sec (21.1)	UN (24)	Ill (56)
	We can propagate most M/H plants by transplanting	Ill (61)	Sec (44)	Sec (15)	Pry (8)
	I know types of chemical fertilizers added to M/H plants	Unv (38)	Unv (30)	Sec (24)	Ill (28)
	I know different methods of drying M/H plants	Ill (I56)	Pri (46)	UN (16)	UN (14)
	I think it's possible to store fresh M/H plants for long period	Ill (44)	Pri (39)	High (11)	High (34)
	I believe there are some projects specialized in planting M/H plants	UN (34)	High (38)	Ill (22)	UN (57)
Education	I believe we must conserve natural M/H plants	UN (81)	Pri (38)	Pry (8)	High (2)
	I believe that over harvesting is the main reason for depletion of M/H plants	Ill (72)	Pri (46)	High (6)	High (13)
	I believe that conserving wild M/H plants help in biodiversity	UN (77)	Ill (18)	Pri (13)	Pri (17)
	I believe that farmers need awareness programs for conserving M/H plants	ill (89)	Pri (32)	High (6)	High (21)
	I get benefits from the information from the extension agent	High (28)	Pri and sec (25)	High (12)	UN (37)
	I consider that the extension agent is the source of agricultural information	Ill (33)	Pri and sec (25)	UN (11)	Ill (39)
	Extension agent provide me in seasonal advices	Sec (25)	Pri (25)	Pri (25)	Ill (25)
	The best way of using M/H plants is boiling	6--10 (24)	1-5 (28)	11-15 (46)	<1 (48)
Farm size	The best way of using M/H plants is soaking	<1 (72)	6-10 (10)	11-15 (31)	>15 (16)
	M/H plants could be propagated by seeds	6-10 (50)	11-15 (39)	1-5 (10)	6-10 (5)
	M/H plants needs partial irrigation 2-3 times /week	>15 (52)	6-10 (33)	6-10 (10)	<1 (5)

Table 3. Cont'd.

	I believe that most M/H plants could be irrigated by drip irrigation	<1 (53)	1-5 (37)	11-15 (15)	11-15 (15)
	I know the way of putting irrigation system	<1 (42)	1-5 (45)	11-15 (39)	11-15 (23)
	I know the maintenance of irrigation system	>15 (45)	>15 (20)	6-10 (33)	<1 (51)
	I know types of chemical fertilizers added to M/H plants	>15 (53)	>15 (20)	1-5 (24)	<1 (42)
	We can add chemical fertilizers through irrigation (fertigation)	>15 (41)	11-15 (39)	6-10 (25)	<1 (36)
	Aphids considered as main insect that attack M/H plants	1-5 (42)	11-15 (33%)	6-10 (14%)	11-15 (15%)
	Root rot considered one of the diseases that attack M/H plants	>15 (20)	>15 (30)	11-15 (42)	<1 (36)
	I know methods of grading and packaging fresh M/H plants	>15 (26)	>15 (32)	11-15 (39)	<1 (39)
	I know different method of drying M/H plants	<1 (60)	6-10 (30)	6-10 (20)	11-15 (17)
	I believe that farmers need awareness programs for conserving M/H	11-15, <! (33)	>15 (35)	11-15 (25)	6-10 (24)
	I visit extension agent continuously	6-10 (38)	11-15 (39)	>15 (20)	<1 (24)
	I get benefits from the information from the extension agent	<1 (49)	11-15 (46)	>15 (14)	>15 (18)
	I consider that the extension agent is the source of agric- information	11-15 (39)	6-10 (43)	>15 (18)	<1, 6-10 (14)
	Extension agent provides me seasonal advices	6-10 (29)	>15 (12)	>15 (25)	<1 (54)
Experience	I know nutritional benefits of M/H	16-20 (39)	16-20 (50)	>20 (9)	>20 (14)
	The best way of using M/H plants is boiling	>20 (22)	6-10 (24)	<5 (23)	6-10 (36)
	I know preparation of soil for planting M/H plants	16-20 (56)	6-10 (40)	<5 (16)	11-15 (5)
	I believe that real production of irrigated M/H plants starts in the second season	>20 (40)	16-20 (44)	<5 (24)	>20 (23)
	I know different method of drying M/H plants	6-10 (39)	6-10 (37)	11-15 (16)	<5 (21)
	I believe that good leaves of M/H plants should be free from diseases	>20 (74)	16-20 (50)	16-20 (11)	11-15 (3)
	I believe that the best way of harvesting wild M/H plants is pulling it out	<5 (19)	6-10 (11)	<5 (28)	>20 (71)
	I believe that protecting M/H plants needs a law	>20 (70)	11-15 (26)	6-10 (16)	>20

and maintenance 52%. the fifth and sixth training need is fertilizer application, 51%, and the soil sterilization, 48%, respectively. the seventh training need is planting methods ,46%; the eighth training need is weed control, 41%; and the ninth training need is soil preparation, 29%.Chi-square test was used in conducting the analysis for determining the relationship between training needs and certain personal characteristics of farmer. The data in Table 5 show only the significant relationship. Males showed that they need higher training in marketing 43%; disease control, 37%; and harvesting, 29%. On the other hand, the study showed that females, 26%, need training in fertilizers application. Work status as contained in Table 6 indicates that training courses are highly required by full time farmers as follows: training courses in diseases identifications and control, 28%; and training courses in irrigation implementation and maintenance, 21%. Regarding the relationship between training needs and educational level of farmers in Table 7, the study indicates that training courses are highly required by farmers holding a university degree as follows: training courses in diseases identifications and control, 19%; training courses in soil preparation and plowing, 10%.

Conclusions

According to the study analysis, more than half of the farmers are highly aware of conservation and production processes of M/H plants, whereas 23% of farmers have low awareness. Furthermore, the analysis of the study concluded that training courses on all aspects of marketing, locally and internationally, training courses on diseases identifications and control, fertilizer use and

Table 4. Knowledge and practice of new managements in ranking order.

Training needs	Percentage
Training courses in marketing (local and international)	72
Training courses for identifying Diseases (pests and diseases, IPM control, methods of using pesticides and safety periods)	65
Training courses in harvesting (harvesting time, method of harvesting, grading, packaging, cooling and storing fresh and dried M/H plants)	53
Training courses in irrigation (irrigation methods, implementing drip irrigation system, maintenance, determining the amount and time of water application)	52
Training courses in fertilizers Applications (types of chemical fertilizers, methods of applying, determining the amount and time of fertilizers application)	51
Training courses in soil sterilization (chemically, solar)	48
Training courses in planting (methods of transplanting, time of planting, spacing, methods of propagation seeds and cuttings)	46
Training courses in weed control (manually, chemically and mechanically)	41
Training courses in soil preparation and plowing (time, methods, number of plowing and types of plows, manure application)	29

Table 5. Knowledge and practice of new managements in association to gender.

Training needs	Gender	Percentage
Training courses in marketing (locally and internationally)	M	43
Training courses in identifying diseases (pests and diseases, IPM control, methods of using pesticides and safety period)	M	37
Training courses in Harvesting (harvesting time, method and harvesting numbers, grading, packaging, cooling and storing fresh and dried M/H)	M	29
Training courses in Fertilizers application (types of chemical fertilizers, methods of applying, determining the amount and time of fertilizers application)	F	26

Table 6. Knowledge and practice of new managements in association with work status of farmers.

Training needs	Work status	Percentage
Training courses in disease control (pests and diseases, IPM control, methods of using pesticides and safety period)	Full time	28
Training courses in Irrigation (irrigation methods, implementing drip irrigation system, maintenance, determining the amount and time of water application	Full time farmer	21

applications, irrigation design, implementation and maintenance, various methods of soil sterilization, planting methods and cultural practices, weed identification and control, harvesting, post harvesting methods, processing and storage are important.

The lack of awareness and knowledge in these aspects will influence the forces that seriously challenge M/H production and collection from the wild. Therefore, studying farmer's awareness and their training-needs will increase production and help in conserving M/H plant in

Table 7. Knowledge and practice of new managements in association with education level of farmers.

Training needs	Education level	Percentage
Training courses regarding the identification of diseases that include (pests and diseases, IPM control, methods of using pesticides and safety periods)	University	19
Training courses in soil preparation and plowing (time, methods, number of plowing and types of plows, manure application)	University	10

Jordan.

RECOMMENDATIONS

From the data of the current investigation, these are the suggested recommendation: Improve knowledge level of medicinal plants program recommended for unschooled farmers that focus on all aspects of production and conservation of (M/H) plants; conducting more programs on fertilizers application targeting framers who own less than 0.1ha and grows M/H plants; execution of programs on pesticides applications and safety measures; advance program targeting the private sector to invest more in the establishment of different types of high quality varieties and healthy seedlings; and more training programs for persona extension to equip them with the latest technologies (harvesting, post harvesting, grading, packaging, marketing and cultural practices) in the production and conservation of M/H plants.

Conflict of Interests

The authors have not declared any conflict of interests.

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- *Journal of Toxicology and Environmental Health Sciences*