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Full Length Research Paper

Phytochemical analysis and ovicidal activity of *Cassia sieberiana*, *Guiera senegalensis* and *Excoecaria grahamii* extracts

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***Cassia sieberiana* DC. (Caesalpiniaceae), *Guiera senegalensis* JF. Gmel (Combretaceae) and *Excoecaria grahamii* (Hochst) Pax. (Euphorbiaceae) are used in traditional medicine in Burkina Faso to treat gastro-intestinal parasites. Standard methods were used for phytochemical screening and to evaluate the *in vitro* anthelmintic activity of the aqueous extracts of the three (3) plants using the egg hatch test against *Haemonchus contortus* eggs. The phytochemical analysis revealed the presence of anthocyanosides, tannins, saponosides, reducing compounds, carbohydrates, flavonoids and triterpenic steroids in the extracts of the root bark of *C. sieberiana* and the leaves of *C. sieberiana*, *G. senegalensis* and *E. grahamii*. All extracts inhibited significantly ($p < 0.05$) the eggs hatch of *H. contortus*. At the concentration of 14.25 mg/mL, the percentages of inhibition varied from 60.33 ± 8.43 to 76.6 ± 4.91%. These results indicate that the studied plants possess anthelminthic activities and thus justify their use for the treatment of gastrointestinal parasites infections.**

Key words: Anthelmintic activity, medicinal plants, phytochemical, *in vitro*, *Haemonchus contortus*, Burkina Faso.

INTRODUCTION

Gastrointestinal parasites infections are public health problem in humans and livestock. In particular, the high prevalence of intestinal nematodes, with an estimated 3.5 billion people being infected every year has been reported (Horton, 2003; Bethony et al., 2006; Grzybek et al., 2016). Thus, more than a quarter (1/4) of the world's population is affected by parasites infections including helminthosis. They are often responsible for nutritional deficiency with heavy consequences for the children, girls of childbearing age and pregnant women (WHO, 2008).

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Indeed, these diseases undermine the intellectual growth, the cognitive development and the school performance in children (Hotez, 2009; WHO, 2015). These gastrointestinal parasites are also responsible for high morbidity, weight loss, poor reproductive status and likely mortality in livestock (Ndao et al., 1995; Wolstenholme et al., 2004; Grzybek et al., 2016). Their control is based on the use of modern anthelmintic drugs (Hounzangbe-Adote et al., 2005; Hoste et al., 2011). However, the excessive use of these anthelmintics leads increasingly to the development of resistance of helminths. Due to this factor and other reasons including the problem of accessibility to the modern drugs at the appropriate time and place and the lack of financial resources (Asase et al., 2008), many people in the developing countries use the plants for their primary health care (Stangeland et al., 2008; Tanner et al., 2011; Asase et al., 2008). Thus, the importance and the use of medicinal plants in countries such as Burkina Faso are no longer to be demonstrated. But, the therapeutic efficacy and the chemical contents of many plants remain to be scientifically documented.

Several medicinal plants are used in Burkina Faso to treat human and animal gastrointestinal parasites (Kaboré et al., 2007). Surveys in four areas of the country have shown that *Cassia sieberiana* DC. (Caesalpiniaceae), *Guiera senegalensis* JF. Gmel. (Combretaceae) and *Excoecaria grahamii* (Hochst) Pax. (Euphorbiaceae) are among the most frequently cited plants for the treatment of gastro intestinal parasitosis (Traoré et al., 2013). Several studies have demonstrated the antimicrobial, analgesic, anti-inflammatory, antihypertensive, anti-venomous, spasmolytic, diuretic and laxative effects of these medicinal plants (Anton and Duquenois, 1968; Kerharo and Adam, 1974). Fall et al. (2004) have demonstrated the anticoccidial effects of the butanol extract of *C. sieberiana*. Trypanocidal properties of the leaves of this plant have also been reported (Hoet et al., 2004). The use of *G. senegalensis* in the treatment of diarrhoea, as febrifuge, antineuralgic, and diuretic have been reported by Kerharo et al. (1948).

This paper reported the results of the chemical screening of the extracts of *C. sieberiana*, *G. senegalensis* and *E. grahamii* used in Burkina Faso against gastro intestinal parasites in human and livestock. The extracts of the plants were also evaluated for their anthelminthic activities using the eggs hatch test on the nematode parasite *Haemonchus contortus*.

MATERIALS AND METHODS

Study species

C. sieberiana DC. (Caesalpiniaceae). Synonyms: *Cassia abbreviata* Oliv. ; *Cassia kotschyana* Oliv

Cassia sieberiana is a small tree branched near the base and can reach 5 m high. The stem bark is fissured and lamellar, blackish in old subjects. The leaves are alternate and composed, 10-30 cm long with 6-12 pairs of opposite leaflets. The inflorescences are long hanging clusters of yellow flowers up to 35-50 cm long. The fruits are long and straight cylindrical pods up to 60 to 80 cm long by 10 to 15 mm in diameter, indehiscent, dark brown or blackish at maturity, persisting for a very long time on the tree. The fruit is transversely partitioned, each lobe containing a seed surrounded by a more or less farinaceous yellow pulp.

C. sieberiana is widespread in West Africa and is very common in all savannah woodlands or shrubs of the Sudanian zones up to the edge of the Guinean forest in Casamance. The plant is widely used in traditional medicine as an analgesic in dysmenorrhea, body pain in humans and in veterinary medicine (Sam et al., 2011). The *in vitro* anthelmintic activity against *Caenorhabditis elegans* of the leaves extract have been reported (Waterman et al., 2010).

Guiera senegalensis J.F. Gmel. (Combretaceae) ; Synonym: *Guiera glandulosa* Sm. *G. senegalensis* is an erect shrub, branched almost from the base with several silvery pubescence branches. The leaves are opposite, oval, softly pubescent on both sides, with black glands below and white hairs giving a general green-gray silvery shade to shrubs. The fruit is elongated, densely and long hairy, silver and pink, radiating like hairy legs of a spider. The plant is semi-evergreen and can reach 3 m high. The species is widespread in Africa throughout the Sudano-Sahelian zone; from Senegal to Cameroon up to Sudan. It is widely distributed in Nigeria, Senegal, The Gambia, Mali, Niger and Burkina Faso where it is locally gregarious and very abundant. *G. senegalensis* is regarded by traditional medicine practitioners in West Africa as a panacea, as its medicinal uses are important and varied (Akuodor et al., 2013). It is used to treat cough, pneumonia, and malaria, gastrointestinal pain, dysentery, diarrhea and used against intestinal worms (Maleš et al., 1998).

Excoecaria grahamii (Hochst) Pax. (Euphorbiaceae); Synonym *Sapium grahamii* (Stapf) Prain

E. grahamii is an African small shrub glabrous, unbranched, up to 60 to 90 cm tall with milky and sticky latex. The stems are herbaceous or sub herbaceous. The stem with lignified base is derived from a tuberous creeping root. The fruit is a three-lobed capsule of 2-2.5 cm diameter, hard, brown, with explosive dehiscence, containing three seeds. The seeds are almost globular, brown to yellow and about 5 mm long. The genus Excoecaria occurs in the tropics of the Old World and the Pacific islands and comprises 35 species. There are about 6 species in the continental Africa and about 5 species in Madagascar. *E. grahamii* is common in savannahs and at the edge of forests, usually on moist soils, from Guinea to Nigeria. The whole plant, the leaves and the latex are used in traditional medicine in Benin, Burkina Faso, Ivory Coast, Niger and Nigeria against skin affections, edema, leprosy, dysentery, guinea worms, and hallucinations. In Burkina Faso, the leaves decoction is known to have purgative effects and is also used to induce abortion (Nacoulma, 1996). The roots latex is used for ritual scarifications and tattooing.

Plants material

The leaves and the roots bark of the three (3) plants were collected in the suburbs of Ouagadougou (savanna zone). The plants *C. sieberiana* and *G. senegalensis* were collected at the beginning of the rainy season (June) in Gampéla, located at 15 km in the East of Ouagadougou. *E. grahamii* was collected during the rainy season (August) at Loumbila at 15 km at the North-East of Ouagadougou. The plant's species were confirmed at the herbarium of the National

Centre for Scientific and Technical Research (CNRST) in Ouagadougou, where specimens are deposited under the numbers of HNBU00179 (*C. sieberiana*), HNBU00252 (*G. senegalensis*) and HNBU01032 (*E. grahamii*).

Eggs of *H. contortus*

The *H. contortus* eggs were collected from adult worms harvested from the stomach of naturally infected goats or sheep. The stomachs were bought from the refrigerated slaughterhouse of Ouagadougou.

Plants extracts

The leaves and the roots bark were washed with water and dried from dust. They were finely powdered. The powders were used to make decoctions for the leaves of *C. sieberiana*, *E. grahamii* and *G. senegalensis* and maceration for the roots barks of *C. sieberiana*. For decoctions, 100 g of plant powder were extracted in 1000 mL of distilled water. The mixture was boiled for 1 h. After cooling, the extract is filtered through fine nylon mesh. The filtrates obtained from *C. sieberiana* (DFCS), from *G. senegalensis* (DFGS) and from *E. grahamii* (DFEG) were centrifuged at 2000 rpm for 10 min. The supernatant obtained from each sample was frozen and then lyophilized.

For extraction, 100 g of powder was mixed with 1000 mL of distilled water and allowed to macerate for 48 h before being filtered as for the decoctions. The filtrate obtained from *C. sieberiana* (MERCS) was frozen and then lyophilized.

For the assay, 150 mg of the dried extracts were diluted in 10 mL of distilled water to get a stock solution which was serially diluted to obtain five different concentrations.

Collection of eggs of *H. contortus*

The method used was that described by Jabbar et al. (2006). For this purpose, the adult worms collected in phosphate buffer solution (PBS; pH = 7.2) were washed with distilled water and crushed lightly in a mortar using a porcelain pestle to release the eggs. The solution obtained was filtered through mesh sieves of various sizes (1 mm and 100 µm). Then, a smaller mesh sieve (38 µm) was used to collect the eggs released by the crushed worms. After several rinses with distilled water, the eggs were recovered in solution and the concentration was adjusted to approximately 1000 eggs/mL of distilled water.

Phytochemical screening of the extracts

The decoctions and the macerate obtained were used for phytochemical tests to identify the chemical groups present in the plants. The characterization methods described by Ciulei (1982) were used.

Egg hatch assay

The egg hatch assay was carried out according to the method described by Coles et al. (2006). 100 µL of the egg suspension adjusted to approximately 1000 eggs/mL was deposited in each well of a multiwell (24 wells) plate. In each well, 1900 µL of the lyophilized extracts were added at concentrations of 0.1, 1, 3, 10 and 15 mg/mL giving final concentrations of 0.095, 0.95, 2.85, 9.50 and 14.25 mg/mL. PBS and albendazole were used as negative and positive controls, respectively. Then, the plate was incubated at the laboratory temperature (32°C) for 48 h. At the end of the incubation, a drop of formalin was deposited in each well to stop the evolution of the eggs. Then, the eggs and died or alive larvae were counted under a microscope (Olympus BH-2, Optical Co. LTD, Japan) at 40 X, to establish the egg hatching rate. The test was carried out in triplicate.

The results were expressed as % inhibition of eggs hatch. For each extract concentration, the % inhibition of eggs hatch was calculated using the modified formula of Coles et al. (1992):

Percentage (%) inhibition of egg hatch = $(1-X_2 / X_1) \times 100$

Where, X_1 = initial number of eggs in the well and X_2 = final number of eggs in the well.

Reference drug

The reference drug, albendazole (tablets, 400 mg) used in this study was bought from a local pharmacy. This drug was chosen due to its availability and also because some authors have used it for *in vitro* trials using different concentrations. The tablets were powdered and the powder was diluted in distilled water to obtain a stock solution with a concentration of 15 mg/mL, which was serially diluted to obtain the same concentrations as with the extracts.

Statistical analysis

For the egg hatch assay, the tests were carried out in triplicate and repeated twice (2) or three (3) times. The statistical analysis was performed with the GraphPad Prism software, version 6.01 (Graph Pad Software Inc., San Diego, CA). Results were expressed as mean ± SEM. Values of 50% inhibitory concentrations (IC_{50}: the concentration capable of inducing 50% inhibition of egg hatching) were determined using a nonlinear regression analysis. The ANOVA was used for the analysis of the data. The differences were considered statistically significant when $p < 0.05$.

RESULTS

Phytochemical analysis

The results of chemical screening of the four (4) extracts from the 3 plants are presented in Table 1. According to these results, anthocyanosides, tannins, saponosides, reducing compounds, oses and polyoses, flavonoids and triterpene steroids were found in all extracts. Thus, the 4 extracts showed approximately the same phytochemical profile except for coumarins which were not detected in the roots bark extract of *C. sieberiana* and the alkaloids salts which were found only in the leaves extract of *G. senegalensis*.

Ovicidal activity

The results of the egg hatch assay, testing of the four (4) extracts of the three (3) plants are presented in Figure 1. All the extracts showed a dose-dependent inhibition of the *H.*

Table 1. Phytochemical composition of four extracts from the three plants [#].

Chemical group	Extracts			
	MERCS	DFCS	DFGS	DFEG
Anthocyanosides	+	++	++	++
Tannins	+	++	++	++
Saponosides	++	++	+	+
Reducing compounds	++	++	++	+
Oses and polyoses	++	++	++	+
Alkaloid salts	ND	ND	+	ND
Flavonoids	±	+	++	+
Triterpenes/ steroids glucosides	++	++	++	++
Coumarins and derivatives	ND	++	++	++

ND = not detected; ± = traces; + = presence; ++ = abundant. [#]Root bark extract of *Cassia sieberiana* (MERCS) and leaves extracts from *Cassia sieberiana* (DFCS), *Guiera senegalensis* (DFGS) and *Excoecaria grahamii* (DFEG).

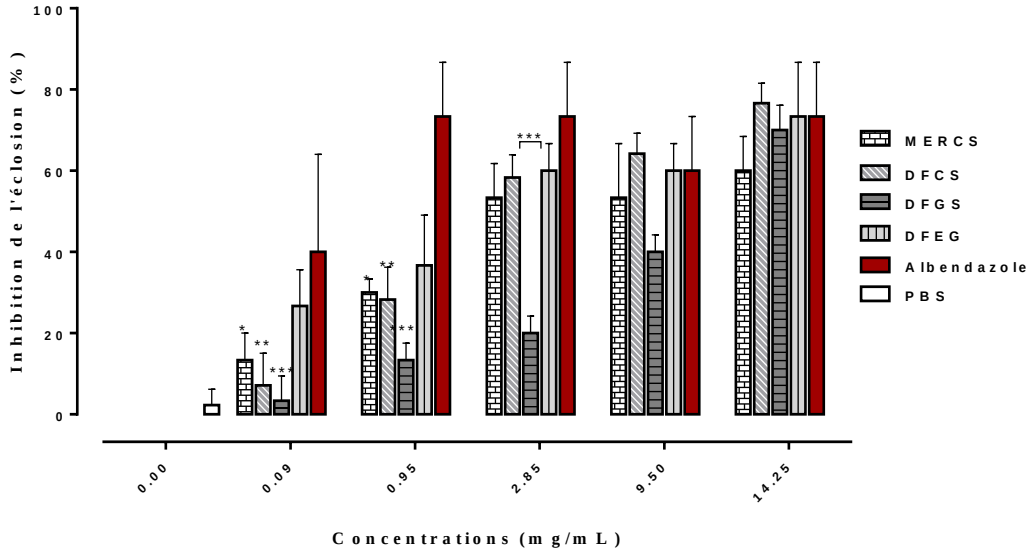

Figure 1. Inhibition of egg hatching (%) for increasing concentrations of four extracts from the three plants[#], the negative control and the positive control (n = 6-9). **, P <0.05; **, p <0.01; ***, p <0.001:* significant compared to the positive control albendazole. [#]Root bark extract of *Cassia sieberiana* (MERCS) and leaves extracts from *Cassia sieberiana* (DFCS), *Guiera senegalensis* (DFGS) and *Excoecaria grahamii* (DFEG).

contortus egg hatching. The values of the % inhibition produced by the extracts and the positive control (albendazole) were statistically different ($p < 0.05$) compared to that of the negative control (PBS) (2.27 ± 0.39 %).

At the concentration of 14.25 mg/mL, the macerate of root bark of *C. sieberiana* (MERCS), the decoction of leaves of *C. sieberiana* (DFCS), the decoction of leaves of *G. senegalensis* (DFGS) and the decoction of leaves of *E. grahamii* (DFEG) showed egg hatch inhibition between 60 and 73%. These effects were similar to the inhibition induced by albendazole (73.34 ± 13.33%) and were significantly different ($p < 0.05$) compared to the negative control (PBS).

Comparison between the four (4) extracts showed that there was no significant difference ($p > 0.05$) between the effect of the leaves extract (DFCS) and the root bark extract (MERCS) of *C. sieberiana.* But the effect induced by DFCS was significantly different ($p < 0.05$) from the effects of DFGS and DFEG. Similarly, MERCS induced a significantly different ($p < 0.05$) effect from that of DFGS. The IC_{50} values for the extracts were determined and are

Table 2. Table 2: The median inhibitory concentration (IC_{50}) values (mg/mL) for four plants extracts from the three plants[#] on *Haemonchus contortus* eggs hatching..

Products	IC_{50} (LI - LS)*
MERCS	1.17 (0.47-2.88)
DFCS	1.05 (0.64-1.73)
DFGS	4.64 (3.07-7.02)
DFEG	0.39 (0.1-1.48)
Albendazole	0.03 (0.0-0.79)

*Values at 95% confidence interval (CI); LI, lower limit of CI; LS, upper limit of CI. [#]Root bark extract of *Cassia sieberiana* (MERCS) and leaves extracts from *Cassia sieberiana* (DFCS), *Guiera senegalensis* (DFGS) and *Excoecaria grahamii* (DFEG).

presented in Table 2.

DISCUSSION

The main chemical groups highlighted in the extracts of *C. sieberiana*, *G. senegalensis* and *E. grahamii* were anthocyanosides, tannins, saponosides, triterpene and steroids. These findings corroborate those of Abubakar *et* al. (2000), Kouamé et al. (2009), Shettima et al. (2012) and Nartey et al. (2012) who reported the presence of these chemical compounds in the three plants. The chemicals found in *G. senegalensis* leaf extracts were alkaloids, saponosides, coumarins, flavonoids, and tannins. These data corroborate the results previously reported. Indeed, the presence of tannins, flavonoids (Abubakar et al., 2000) and alkaloids (Fiot et al., 2005) was revealed in leaves extracts of this plant. Nacoulma (1996) also reported the presence of these constituents in the leafy stems of *G. senegalensis*. In addition to tannins, our results show the presence of saponosides and triterpene/steroids in the leaves and the roots bark of *C. sieberiana*. These results are in agreement with those of Tamboura et al. (2005) which revealed the presence of tannins and saponosides in this plant. These active substances probably explain the current uses of the three plants by traditional healers in the treatment of many human and animal diseases including parasitic infections (Nacoulma, 1996). Indeed, the therapeutic properties of these secondary metabolites have been reported (Cowan, 1999). According to Waterman et al. (2010), tannins and other phenolic compounds possess proteins coagulant properties that would give rise to a broad spectrum of vermicidal activities. It is generally considered that the anthelminthic effects of forage containing tannins are related to their content of condensed tannins (Hoste et al., 2011). Some *in vitro* results suggest also that secondary metabolites such as flavonoid and alkaloïds (Molan *et al.*, 2003;; Barrau et al., 2005; Brunet and Hoste, 2006; Eguale et al., 2008; Chagas et al., 2008; Muthee et al., 2011), saponins (Ademola and Eloff, 2010; Waterman et al., 2010) and terpenoids (Marley et al., 2006; Githiori et al., 2003) may possess some anthelminthic properties. The dose-dependent relationship between the concentration of tannins and/or flavonoid compounds and the anthelminthic activity has been demonstrated under both *in vitro* (Molan et al., 2002; Brunet and Hoste, 2006) and in vivo conditions (Hoste et al., 2011; Brunet et al., 2007; Terrill et al., 2009).

The high egg hatching inhibition (> 60%) induced by the extracts at 14.25 mg/mL and the few inhibition rate (≈ 2.27%) with PBS (negative control), confirm that changes in egg hatch observed with the extracts, result from the ovicidal action of these substances. They induced an inhibition of the egg hatching in the nematode parasite *H. contortus.* Thus, they possess *in vitro* anthelmintic properties.

These results showed clearly that the leaves extract of *E. grahamii* (IC_{50} = 0.39 mg/mL) was more active than the other extracts and 10 times less active than the positive control, albendazole (IC_{50} = 0.03 mg/mL). Based on the IC_{50} values (Table 2), the extracts and the positive control could be classified, in order of power, as follows: albendazole > DFEG > DFCS > MERCS > DFGS. This ovicidal activity could support the use of these plants by traditional healers against gastrointestinal parasites and the chemical groups found in the plant's extracts should be the basis of the biological activities attributed to these plants, including their anthelminthic properties (Paolini et al., 2003). It has been reported that the active molecules with ovicidal activity penetrate the eggshell and prevent its development or paralyze the L_1 larval stage inside the egg (Wabo et al., 2011). The four extracts could have exerted their ovicidal effect through this mechanism. Thus, the hatching process could have been inhibited by active compounds such as nitrogen-containing compounds which are known to have ovicidal properties (Maciel et al., 2006). However, more investigation is needed to identify the active components responsible for this action.

Conclusion

The results from this study revealed that the extracts from *C. sieberiana*, *G. senegalensis* and *E. grahamii* inhibit the egg hatching in *H. contortus* and could be a potential source of anthelmintics. The activity of these extracts can be attributable to the secondary metabolites such as tannins, flavonoids, alkaloids, steroids, terpenoids and/or saponins or to the combined interaction of these components. However, further studies like larvicidal assays and *in vivo* trials should be carried out to confirm the efficacy of these plant species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abubakar MS, Sule MI, Pateh UU, Abdurahman EM, Haruna AK, Jahun BM (2000). In vitro snake venom detoxifying action of the leaf extract of *Guiera senegalensis*. J. Ethnopharmacol. 69(3):253-257.

Ademola IO, Eloff JN (2010). *In vitro* anthelmintic activity of *Combretum molle* (R. Br. ex G. Don) (Combretaceae) against *Haemonchus contortus* ova and larvae. Vet. Parasitol. 169(1):198-203.

Akuodor GC, Essien AD, David-Oku E, Chilaka KC, Akpan JL, Ezeokpo B, Ezeonwumelu JOC (2013). Gastroprotective effect of the aqueous leaf extract of *Guiera senegalensis* in Albino rats. Asian Pac. J. Trop. Med. (2013):771-775.

Anton R, Duquenois P (1968). L'emploi des Cassias dans les pays tropicaux et subtropicaux examiné sous quelques-uns des constituants chimiques de ces plantes médicinales. Pltes Méd. Phytother. 2:225-268.

Asase A, Kokubun T, Grayer RJ, Kite G, Simmonds MSJ, Oteng-Yeboah AA, Odamtten GT (2008). Chemical constituents and antimicrobial activity of medicinal plants from Ghana: *Cassia sieberiana*, *Haematostaphis barteri*, *Mitragyna inermis* and *Pseudocedrela kotschyi*. Phytother. Res. 22(8):1013-1016.

Barrau E, Fabre N, Fouraste I, Hoste H (2005). Effects of bioactive compounds from Sainfoin (*onobrychis viciifolia* Scop.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. Parasitol. 131(4):531-538.

Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ (2006). Soil-transmitted helminth infections: Ascariasis, trichuriasis, and hookworm. Lancet 367:1521-1532.

Brunet S, Aufrere J, El Babili F, Fouraste I, Hoste H (2007). The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of a tannin- rich plant extract (sainfoin) both *in vitro* and *in vivo*. *Parasitol.* 134(9):1253-1262.

Brunet S, Hoste H (2006). Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. J. Agric. Food Chem 54(20):7481-7487.

Chagas ACS, Vieira LS, Freitas AR, Araïjo MRA, Araïjo-Filho JA, Araguyo WR, Navarro AMC (2008). Anthelmintic efficacy of neem (*Azadirachta indica* A. Juss) and the homeopathic product Fator Vermes® in Morada Nova sheep. Vet. Parasitol. 151(1):68-73.

Ciulei I (1982). Practical manuals on the industrial utilization of chemical and aromatic plants. Methodology for analysis of vegetable drugs. Ed. Ministry of Chemical Industry, Bucharest, p. 67.

Coles GC, Bauer C, Borsgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller PJ (1992). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44:35-44.

Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A, Taylor MA, Vercruysse J (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 136(3):167-185.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4):564-82.

Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E (2008). *Haemonchus contortus*: *In vitro* and *in vivo* anthelmintic activity of aqueous and hydro-alcoholic extracts of *Hedera helix*. Exp Parasitol. 116(4):340-345.

Fall AD, Gbati AOB, Diatta W, Sow B, Gueye F, Bassene E, Pangui L (2004). Etude de la fraction anticoccidienne de l'extrait de racines de *Cassia sieberiana* D.C. (Caesalpiniaceae) chez le lapin. J. Pharm. Méd. Trad. Afr. 13:49-55. Available at : http://webcache.googleusercontent.com/search?q=cache:http://greenstone.lecames.org/collect/revueph1/index/assoc/HASH4b8c.dir/13-049-055.pdf

Fiot J, Sanon S, Azas N, Mahiou V, Jansen O, Angenotc L, Balansard G, Ollivier E (2005). Phytochemical and pharmacological study of roots and leaves of *Guiera senegalensis* J.F. Gmel (Combretaceae). J. Ethnopharmacol. 106 (2):173-178.

Githiori J B, Höglund J, Waller PJ, Baker RL (2003). The anthelmintic efficacy of the plant *Albizia anthelmintica*, against the nematode parasite *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* in mice. Vet. Parasitol. 116(1):23-34.

Grzybek M, Kukula-Koch W, Strachecka A, Jaworska A, Phiri AM, Paleolog J, Tomczuk K (2016). Evaluation of anthelmintic activity and composition of pumpkin (*Cucurbita pepo* L.) seed extracts—*in vitro* and *in vivo* studies. Int. J. Mol. Sci. 17 (9):1456-1467.

Hoet S, Opperdoes F, Brun R, Adjakidj V, Quetin-Leclercq J (2004). *In vitro* antitrypanosomal activity of ethnopharmacologically selected Beninese plants. J. Ethnopharmacol. 91(1):37-42.

Horton J (2003). Human gastrointestinal helminth infections: Are they now neglected diseases? Trends Parasitol. 19(11):527-531.

Hoste H, Manolaraki F, Brunet S, Arroyo LC, Martínez-Ortiz de Montellano C, Sotiraki S, Torres AF (2011). The anthelmintic properties of tannin-rich legume forages: from knowledge to exploitation in farm conditions. *In*: Ranilla M.J. (ed.), Carro M.D. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). Challenging strategies to promote the sheep and goat sector in the current global context. Zaragoza : CIHEAM / CSIC / Universidad de León / FAO. p. 295-304 (*Opt. Méditerranéennes : Série A. Séminaires Méditerranéens*; n° 99). Available at: http://om.ciheam.org/om/pdf/a99/00801571.pdf

Hotez PJ (2009). One world health: Neglected Tropical Diseases in a Flat World. PLoS Negl. Trop. Dis. 3:e405.

Hounzangbe-Adote M, Paolini V, Fouraste I, Moutairou K, Hoste H. (2005). *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. Res. Vet. Sci. 78(2):155-160.

Kaboré A, Tamboura HH, Belem AMG, Traoré A (2007). Traitements ethno-vétérinaires des parasitoses digestives des petits ruminants dans le plateau central du Burkina Faso. Int. J. Biol. Chem. Sci. 1(3):297-304.

Kerharo J, Adam JG (1974): Pharmacopée sénégalaise traditionnelle: Plantes médicinales et toxiques. Edition Vigot et frères, Paris.

Kerharo J, Bouquet A, Heintz R (1948). Le Wilinwig-a des Mossi (*Guiera senegalensis*, Lam.), ses usages thérapeutiques indigènes et son application au traitement des diarrhées cholériformes. Acta Trop. 5:345.

Kouamé J, Gnoula C, Palé E, Bassolé H, Guissou IP, Simporé J, Nikiéma J -B (2009). Etude des propriétés cytotoxiques et anti-radicalaires d'extraits de feuilles et de galles de *Guiera senegalensis* J. F. Gmel (Combretacae). Sci. et technique-Sciences de la santé, pp. 9-23.

Maciel MV, Morais SM, Bevilaqua CML, Camurça-Vasconcelos ALF, Costa CTC, Castro CMS (2006). Ovicidal and larvicidal activity of *Melia azedarach* extracts on *Haemonchus contortus*. Vet. Parasitol. 140(1):98-104.

Maleš Ž, Medic-Šarić M and Bucar F (1998). Flavonoids of *Guiera senegalensis* J. F. Gmel.Thin-layer Chromatography and Numerical Methods. Croatica Chemica Acta 71(1):69-79.

Marley CL, Cook R, Barrett J, Keatinge R, Lampkin NH (2006). The effects of birdsfoot trefoil (*Lotus corniculatus*) and chicory (*Cichorium intybus*) when compared with perennial ryegrass (*Lolium perenne*) on ovine gastrointestinal parasite development, survival and migration. Vet. Parasitol. 138(3):280-290.

Molan AL, Meagher LP, Spencer, PA, Sivakumaran S (2003). Effect of flavan-3-ols on *in vitro* egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis* . Int. J. Parasitol. 33(14):1691-1698.

Molan AL, Waghorn GC, McNabb WC (2002). Effect of condensed tannins on egg hatching and larval development of *Trichostrongylus colubriformis in vitro*. Vet. Rec. 150:65-69.

Muthee JK, Gakuya DW, Mbaria JM, Kareru PG, Mulei CM, Njonge FK (2011). Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya. J. Ethnopharmacol. 135(1):15-21.

Nacoulma OG (1996). Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso : cas du plateau central. Thèse de Doctorat ès Sciences Naturelles. Faculté des Sciences et Techniques, Université de Ouagadougou, 320p.

Nartey ET, Ofosuhene M, Kudzi W, Agbale CM (2012). Antioxidant and gastric cytoprotective prostaglandins properties of *Cassia sieberiana* roots bark extract as an anti-ulcerogenic agent. BMC Complem. Altern. Med. 12(1):1-10.

dao M, Belot J, Zinsstag J, Pfister K (1995). Épidémiologie des helminthoses gastro-intestinales des petits Ruminants dans la zone sylvo-pastorale au Sénégal. Vet. Res. 26(2):132-139.

Paolini V, Frayssines A, De La Farge F, Dorchies P, Hoste H (2003). Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. Vet. Res. 34(3):331-339.

Sam GH, Mensah MLK and Nyakoa-Ofori N (2011). Pharmacognostic studies and standardization of *Cassia sieberiana* roots. Pharmacogn. J. 3(21):12-17.

Shettima YA, Tijjani MA, Karumi Y, Sodipo OA (2012). Phytochemical and anti-diarrhoeal properties of methanol root extract of *Guiera senegalensis* J.F.Gmel. *Inter. Tes. J. Pharm.*, 3 (11):61-65. Available at: http://www.irjponline.com/admin/php/uploads/1472_pdf.pdf

Stangeland T, Dhillion S S, Reksten H (2008). Recognition and development of traditional medicine in Tanzania. J. Ethnopharmacol. 117(2):290-299.

Tamboura HH, Bayala B, Lompo M, Guissou IP, Sawadogo L, (2005). Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of *Holarrhena floribunda* (G. Don) Durand and Schinz., *Leptadenia hastata* (Pers.) Decne and *Cassia sieberiana* (DC) used by veterinary healers in Burkina Faso. Afr. J. Tradit. Complem. Altern. Med., 2(1):13-24.

Tanner S, Chuquimia-Choque ME, Huanca T, McDade TW, Leonard WR, Reyes-García V (2011). The effects of local medicinal knowledge and hygiene on helminth infections in an Amazonian society. Soc. Sci. Med. 72(5):701-709.

Terrill TH, Dykes GS, Shaik SA, Miller JE , Kouakou B, Kannan G, Burke JM, Mosjidis JA, (2009). Efficacy of *sericea lespedeza* hay as a natural dewormer in goats: Dose titration study. Vet. Parasitol. 163(1):52-56.

Traoré A, Ouedraogo S, Lompo M, Traoré S, Somé N, Guissou IP (2013). Ethnobotanical survey of medicinal plants used to treat gastrointestinal parasites in human and livestock in four geographic areas of Burkina Faso (West Africa). Arch. Appl. Sci. Res. 5(6):172-177.

Wabo PJ, Kenne TF, Mpoame MP, Tedonkeng E, Bilong Bilong CF (2011). *In vitro* activities of acetonic extracts from leaves of three forage legumes (*Calliandra calotyrsus*, *Gliricidia sepium* and *Leucaena diversifolia*) on *Haemonchus contortus*. Asian Pac. J. Trop. Med. 4(2):125-128.

Waterman C, Smith RA, Pontiggia L, Dermarderosian A (2010). Anthelmintic screening of Sub-Saharan African plants used in traditional medicine. J. Ethnopharmacol. 127(3):755-759.

Wolstenholme AJ, Fairweather I, Prichard R, von Samson-Himmelstjerna G, Sangster NC (2004). Drug resistance in veterinary helminths. Trends Parasitol. 20(10):469-476.

World Health Organization (WHO) (2008). Chimio prévention des helminthiases chez l'homme: utilisation coordonnée des médicaments anthelminthiques pour les interventions de lutte : manuel à l'intention des professionnels de la santé et des administrateurs de programmes. / D.W.T. Crompton; Genève, 64p.

World Health Organization (WHO) (2015). Relevé épidémiologique hebdomadaire, 18 Décembre 2015, N° 51/52: 701-712. Available at: http://www.who.int/wer/2015/wer9051_52.pdf

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Gastro-protective potential of *Artemisia parviflora*on aspirin induced gastric ulcers in albino rabbits

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Artemisia parviflora **Roxb.(Asteraceae) has medicinal properties. This study evaluates the gastroprotective potential of *A. parviflora* seeds against aspirin induced ulcers in albino rabbits.Thirty-six rabbits were randomly divided into 6 equal groups (n = 6). Group 1 was control; group 2 received aspirin for 14 days; group 3 received omeprazole + aspirin for 14 days; groups 4, 5, and 6 received *A. parviflora* seed powder 250, 500, and 750 mg/kg, respectively along with aspirin, for 14 days. Total oxidant status (TOS), totalantioxidant capacity (TAC), malondialdehyde (MDA), and catalase (CAT) were determined to check the gastric damage. Ulcer score, gastric volume, gastric pH, and total acid output was also measured to determine gastroprotective potential of *A. parviflora*. *A. parviflora* seed powder exhibit gastroprotective potential with significant reduction in the ulcer score, acid output, and gastric volume while the pH of gastric mucosa increases significantly at the dose of 750 mg/kg when compared to aspirin treated group. Biochemical analysis showed a significant increase in TAC and CAT activity while it showed significant decrease in the levels of TOS and MDA which indicate reduction in gastric damage.*A. parviflora* seed powder proved to be gastroprotective at 250, 500 and 750 mg/kg with gastric protection of 47.5, 58.1, and 73.5%, respectively. It also has potent antioxidant properties.**

Key words: Antiulcer, ulcer score, gastric volume, gastric pH.

INTRODUCTION

Stomach stores food, possesses antibacterial action, and secretes gastric juices (Baumgart and Sandborm, 2007) while gastric ulcer is a rupture in the normal gastric mucosa that extends throughout the muscularis mucosa. Gastric ulcer could be divided into two common types according to location, ulcerative colitis (lower) and peptic ulcer (upper). Peptic ulcer develops when aggressive and protective factors are imbalanced. *Helicobacter pylori*, non-steroidal anti-inflammatory drugs (NSAIDs), pepsins, hydrochloric acid (HCl) and bile acid are the aggressive factors (Malairajan et al., 2008). NSAIDs weaken the protective mucous layer of the stomach wall and increase the secretion of HCl (Awaad et al., 2013). Epigastric pain is the predominant symptom of uncomplicated gastric ulcer along with more dyspeptic symptoms such as early satiety, bloating, nausea and fullness. In duodenal ulcer patient, epigastric ache occurs frequently during the night or the state of fasting and is usually relieved by acid-

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neutralising agents or food intake. Heartburn and erosive esophagitis are also reported in these patients (Gisbert and Pajares, 2003).

The long term use of NSAIDs (aspirin) mainly inhibit the prostaglandin (PG) and cyclooxygenase (COX) enzyme which causes damage in gastric mucosa with production of free redicals (Baigent et al., 2009). The amount of serum lipid peroxidation (LPO) improved during the ulcer formation while catalase (CAT) and superoxide dismutase (SOD) levels decreased. There is an affirmative relationship that exists between the oxidative stress produced by free radicals, gastric ulcer and gastric carcinoma (Tandon et al., 2004). Most of the existing gastroprotective drugs operate on the offensive aspects neutralizing acid discharge like antacids, H2 receptor blockers like ranitidine, anticholinergics like pirenzepine and proton pump inhibitors like omeprazole that interfere with acid secretion (Ahmad et al., 2006). Proton pump inhibitors are the main synthetic antiulcer class of drugs that reduce the production of acid. Alternative therapies have been developed for the management of GI ulcers and herbal medicines are most important source for GI ulcer management.

Artemisia parviflora Roxb. is an aromatic shrub, leaves are sessile, about 40-100 cm in height and found throughout Pakistan at high elevations. Although phytoconstituents of *A. parviflora* have medicinal importance, its gastroprotective effect has not been studied. *A. parviflora*is commonly used for skin diseases, cuts and wounds this could be due to the different phytochemical constituents of *A. parviflora*. These constituents have inhibitory effect on carcinogenesis in humans and also shows antioxidant activity (Ahuja et al., 2011). Vital oils of *Artemisia* species are extensively utilized for a variety of medicinal uses such as antibacterial, fungicidal, antiviral,nematicidal and antimalarial (Ahmad et al., 2006).In present study antiulcer effect of *A. parviflora* was evaluated against aspirin induced ulcers in albino rabbits.

MATERIALS AND METHODS

Experimental animals

Thirty-six male adult albino rabbits weighing 1.63 ± 0.25 kg were selected for the current studyand divided into six equal groups (n = 6). Animals were housed in the experimental animal room, Department of Physiology and Pharmacology, University of Agriculture, Faisalabad (UAF), Pakistan at temperature 22 ± 2ºC, humidity 65 to 70% and a 12 h light/dark cycles for a week before the start of the experiment and during the experiment. Animals were provided with standard feed and water *ad libitum*. Further, the institutional ethical committee of UAF, Pakistan approved all procedures adopted in this study.

Plant materials

A. parviflora seeds were purchased from the herbal dealers of Faisalabad, Pakistan. The plant material was authenticated by Dr. Mansoor Hameed, Department of Botany, Faculty of Sciences, UAF, Pakistan. A voucher specimen of *A. parviflora* seeds have been deposited in the Herbarium maintained by Department of Botany, UAF, Pakistan. The samples were preserved in the Pharmacology laboratory of theDepartment of Physiology and Pharmacology, UAF, Pakistan. The material was finely powdered with the help of a special electrical grinder. This process was done withthe precaution that the temperature did not rise up to 40ºC. This powder was passed through mesh sieve no. 200 and then stored in an airtight container for further experimental use.

Treatment protocols

All the rabbits were divided into six equal groups (n = 6). Group 1 served as untreated control and received normal diet throughout the experiment, group 2 received aspirin (Disprin®; Reckitt Benckiser(Pakistan) Ltd. Karachi, 150 mg/kg orally for 14 days (Herbert et al., 2011). Group 3 received omeprazole (Omega®; Ferozsons Laboratoies Ltd. Karachi, 20 mg/kg) + aspirin (150 mg/kg) for 14 days (Maity et al., 2003). Groups 4, 5, and 6 received *A. parviflora* seed powder 250, 500, and 750 mg/kg, respectively along with aspirin 150 mg/kg for 14 days. Five ml distilled water was used to dilute the *A. parviflora* seeds powder before administration to the rabbits.

Surgical procedures

The animals were fasted for at least 24 h before the surgical procedure. On the 14th day of theexperiment these animals were sacrificed by decapitation. After this, the stomach was dissected out and transferred in to small tubes. The stomach was cut along the greater curvature and the contents were collected into small tubes for biochemical parameters. These gastric contents were subsequently centrifuged at 3000 rpm for 5 min. The supernatant was separated and its volume was expressed as ml/100 g body weight.

Blood sampling

Blood samples were collected at 14th day of experimental treatments. The samples were allowed to clot for 20 min at refrigeration temperature and then centrifuged for 5 min at 4000 rpm to separate serum. Serum was stored at -4ºC till the estimation of different antioxidant parameters.

Acid output

The acid output was calculated by titrating the supernatant fluid collected from the stomach with 0.05N NaOH. Acidity was expressed as molEq/l/100 g of body weight (Maity et al., 2003).

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality}}{0.1} \times 100$$

Ulcer index

The number of ulcers was noted and the severity was determined with the following scores: Normal coloration (0.0), Red coloration (0.5), Spot ulcer (1.0), Hemorrhagic stress (1.5), Deep ulcers (2.0), Perforation (3.0). Ulcer index (UI) was calculated using the formula (Vogel, 2002).

$$\text{UI} = \frac{\text{US} + \text{UN} + \text{UP}}{10}$$

Table 1. Mean values of ulcer score, ulcer index and curative ratio after 14 days of treatment with per-oral drugs and *A. parviflora* seed powder in rabbits.

Group	Dose (mg/kg)	Ulcer score	Ulcer index	Curative ratio %
1	Routine feed	0.08 ± 0.08^{c}	0.00	
2	150	2.35 ± 0.35^{a}	13.9	
3	20	0.67 ± 0.24^{c}	3.25	76.6
4	250	1.78 ± 0.20^{ab}	7.29	47.5
5	500	1.07 ± 0.30^{bc}	5.82	58.1
6	750	0.54 ± 0.24^{c}	3.67	73.5

Similar letters on means (n = 6) in a column are statistically non-significant ($p \geq 0.05$), 1 = control, 2 = Aspirin, 3 = Omeprazole + Aspirin, 4 = Aspirin +250 mg/kg *A. parviflora* seed powder, 5 = Aspirin + 500 mg/kg *A. parviflora* seed powder, 6 = Aspirin +750 mg/kg *A. parviflora* seed powder.

Table 2. Mean values of Acid output, pH and gastric volume after the 14 days of treatment with per-oral drugs and *A. parviflora* seed powder in rabbits.

Group	Dose (mg/kg)	Acid output (molEq/l/100 g)	pH	Gastric volume (ml/100 g)
1	Routine feed	26.5 ± 0.27^{e}	2.05 ± 0.06^{b}	20.9 ± 0.34^{e}
2	150	39.7 ± 0.16^{a}	0.87 ± 0.16^{a}	35.1 ± 0.10^{a}
3	20	28.9 ± 0.27^{d}	4.47 ± 0.21^{d}	24.9 ± 0.15^{d}
4	250	35.2 ± 0.19^{b}	1.13 ± 0.20^{a}	32.1 ± 0.29^{b}
5	500	31.7 ± 0.25^{c}	3.01 ± 0.24^{c}	29.8 ± 0.22^{c}
6	750	29.4 ± 0.25^{d}	4.10 ± 0.22^{d}	25.6 ± 0.23^{d}

Similar letters on means (n = 6) in a column are statistically non-significant ($p \geq 0.05$), 1 = control, 2 = Asprin, 3 = Omeprazole + Aspirin, 4 = Aspirin +250 mg/kg *A. parviflora* seed powder, 5 = Aspirin + 500 mg/kg *A. parviflora* seed powder, 6 = Aspirin +750 mg/kg *A. parviflora* seed powder.

Where, US = mean severity of ulcer score. UN = average number of ulcers per animal. UP = percentage of animals with ulcer incidence.

Curative ratio

The curative ratio from the ulcer was calculated for the treated groups by using the following equation.

$$\text{Percentage (\%)} = \frac{[\text{CUI-TUI}]}{\text{CUI}} \times 100$$

Where, CUI = ulcer index of control groups. TUI = ulcer index of treated groups.

Biochemical examination

Malondialdehyde (MDA) was determined according to the method developed by Ohkawa et al. (1979). Enzymatic activity of enzyme catalase (CAT) was measured by method of Goth (1991). The total oxidant status (TOS) and the total antioxidant capacity (TAC) in serum was measured by methods developed by Erel (2004, 2005) using spectrophotometer.

Statistical analysis

The values were expressed as mean ± SE. Statistical analysis was performed by one way analysis of variance (ANOVA) at 5% level of significance (Steel et al., 1997).

RESULTS

The seeds powder of *A. parviflora* 250 to 750 mg/kg, given orally once daily for fourteen days showed dose dependent protective effect against gastric ulcer induced by aspirin. *A. parviflora* at the dose rate of 750 mg/kg significantly protected the animal and healed ulcer after fourteen days of treatment.

Mean ulcer score of group 2 was 2.35 that increased significantly ($p<0.05$) after administration of aspirin, while it significantly decreased for group 3 as 0.67 which was treated with omeprazole + aspirin and also it was decreased for group 6 which was treated with the highest dose of test plant, having value of mean ulcer score 0.54.

Ulcer index also showed the similar pattern of results as that of ulcer score. After fourteen days of the treatment ulcer index for groups 1, 2, 3, 4, 5, and 6 was 0.00, 13.9, 3.25, 7.29, 5.82, and 3.67, respectively. Group 3 (Omeprazole + aspirin) and highest plant dose in group 6 showed the significant ($p < 0.05$) reduction in ulcer index. The percent curative ratio of *A. parviflora* seed powder at 250, 500, and 750 mg/kg was 47.5, 58.1, and 73.5%, respectively as shown in Table 1.

Total acid output (molEq/l/100 g body weight) of rabbits in after 14 days are shown in Table 2. The mean values for acid output showed that the aspirin increased the

Table 3. Mean values of TOS, TAC, MDA, and CAT after the 14 days of treatment with per-oral drugs and *A. parviflora* seed powder in rabbits.

Groups	Dose (mg/kg)	TOS (µmol/l)	TAC (mmol/l)	MDA (nmol/l)	CAT (KU/l)
1	Routine feed	3.69 ± 0.09^{d}	3.06 ± 0.25^{c}	3.34 ± 0.05^{d}	10.4 ± 0.19^{a}
2	150	7.03 ± 0.17^{a}	0.93 ± 0.01^{a}	7.97 ± 0.15^{a}	4.99 ± 0.39^{c}
3	20	3.65 ± 0.05^{d}	2.98 ± 0.15^{c}	3.5 ± 0.09^{d}	9.61 ± 0.28^{a}
4	250	5.94 ± 0.36^{b}	1.35 ± 0.14^{ab}	6.92 ± 0.15^{b}	5.9 ± 0.19^{c}
5	500	4.98 ± 0.31^{c}	1.79 ± 0.20^{b}	5.13 ± 0.32^{c}	7.71 ± 0.26^{b}
6	750	3.63 ± 0.06^{d}	2.78 ± 0.14^{c}	3.26 ± 0.05^{d}	9.23 ± 0.21^{a}

Similar letters on means (n = 6) in a column are statistically non-significant ($p \geq 0.05$), 1 = control, 2 = Aspirin, 3 = Omeprazole + Aspirin, 4 = Aspirin + 250 mg/kg *A. parviflora* seed powder, 5 = Aspirin + 500 mg/kg *A. parviflora* seed powder, 6 = Aspirin + 750 mg/kg *A. parviflora* seed powder.

acidity in group 2 having mean value 39.7 molEq/l/100 g as compared to the group 1 which has mean value for acidity 26.5 molEq/l/100 g, while the groups treated with *A. parviflora* seed powder showed significant results at dose 500 and 750 mg/kg as 31.7 and 29.4 molEq/l/100 g, respectively.It was also observed that pH was decreased markedlyin aspirin (group 2) treated rabbits compared to group 1, from 2.05 to 0.87. Omeprazole (group 3) enhanced pH (4.47) of gastric mucosa. Our test plant also increased the pH of gastric mucosa which was 4.10 at the dose of 750 mg/kg. It was observed from the result that gastric volume also increased after the administration of aspirin (35.1) while administration of omeprazole and *A. parviflora* (750 mg/kg) significantly decreased mean gastric volume 24.9 and 25.6, respectively presented in Table 2.

Results of the study showed that mean values of TAC for the group 1 was 3.06 mmol/l. TAC decreased (0.93 mmol/l) with ulcer (group 2) production in stomach by the use of aspirin while it significantly ($p< 0.05$) increases upto normal values for groups 3 and 6 as 2.98 and 2.78 mmol/l, respectively presented in Table 3.

The mean values of TOS of group 1 was 3.69 µmol/l. TOS increased (7.03 µmol/l) with ulcer production in stomach by the use of aspirin while it significantly ($p< 0.05$) decrease up to normal values for groups 3 and 6 as 3.65 and 3.63 µmol/l, respectively when compared with aspirin treated group presented in Table 3.

Results of the current study also demonstrated that the mean values of MDA activity were increased (from 3.34 to 7.97 nmol/l) when aspirin was used alone for the production ofan ulcer in the stomach. The mean values decrease significantly ($p< 0.05$) for groups 3, 4, 5, and 6 as 3.5, 6.92, 5.13, and 3.26, respectively provided in Table 3.

The mean values of CAT activity decreased up to 4.99 KU/l when aspirin was used alone for the production of anulcer inthe stomach. This value is significantly ($p < 0.05$) decreased froma normal value, that is, 10.4 KU/l and its value seems to increase significantly when treated with omeprazole (9.61 KU/l). It also had a normal range of values for CAT activity whenthe plant was used atthe highest dose as in group 6 (9.23 KU/l) which presented in Table 3.

DISCUSSION

Gastric ulcer is a break in the normal gastric mucosa of the stomach that extends throughout the muscularis mucosa into the submucosa or deeper. In ulcer condition erosions are formed and superficial epithelium of mucosa is loosened. In the alimentary tract ulcer may occur everywhere. PGs play a significantprotective role in the stomach by stimulating the synthesis and secretion of mucus and bicarbonate, increasing mucosal blood flow and promoting epithelial proliferation. The major mechanism via NSAIDs cause ulcers is the inhibition of PGs by the inhibition of COX, which isa key enzyme in the bio-synthesis of PGs (Hamid et al., 2012). So to avoid all these adverse reactions of the drugs, herbal remedies should be given to the ulcer patient to cure the ulcer. Many natural products in plants have multifunctional molecules that protect them from infections of bacteria, viruses and other microorganisms. For this reason, we evaluated the antiulcer activity ofthe graded dose of*A. parviflora* seed powder in albino rabbits.

In the present study, results demonstrated that ulcer scores were significantly increased in animals treated with aspirin. Administration of synthetic antiulcer drug, omeprazole at dosea of 20 mg/kg along with aspirin significantly reduced the ulcer scores in comparison with aspirin treated rabbits. Concomitant administration of *A.parviflora* seed powder atthe dose rate of 250, 500, and 750 mg/kg along with aspirin significantly reduced the ulcer scores. The mean value of *A. parviflora* at dose rate of 750 mg/kg was not significantly different from omeprazole. The results of our study conicide with other studies (Aslam et al., 2013; Begum et al., 2014).

pH of gastric secretions was measured in the current study. Administration of aspirin significantly reduced the pH of gastric mucosa as described in previous studies

(Aslam et al., 2013). The administration of omeprazole along with aspirin significantly enhanced the pH of gastric mucosa. Concomitant administration of *A. parviflora* seed powder with aspirin significantly enhanced the pH of gastric secretions at three different doses 250, 500, and 750 mg/kg.

A. parviflora ata dose rate of 750 mg/kg significantly enhanced the pH and it produced similar results as synthetic antiulcer drug omeprazole. Aspirin causes the gastric damage by making the stomach pH more acidic which increases the acidity of the gastric mucosa by enhancing the concentration of hydrogen ions. These results are parallel as described in previous studies (Alsabri et al., 2013; Aslam et al., 2015).

The gastric volume was significantly increased in groups 2. Aspirin enhances the acid secretions in gastric mucosa due to its acidic nature which enhances the volume of gastric secretions. Administration of omeprazole along with aspirin significantly reduces the gastric volume. Concomitant administration of *A. parviflora* along with aspirin significantly reduced the gastric volume at 250, 500, and 750 mg/kg. *A. parviflora* at a dose of 750 mg/kg significantly reduced gastric volume and its results were statistically similar with synthetic antiulcer drug omeprazole. The above mentioned results are consistent with previous research studies on aspirin induced gastric ulcer (Goswami et al., 2011). Pretreatment with an oral dose of *A. parviflora* could partially decrease the ulcer index and permit the healing of gastric lesions induced by the administration of aspirin.

The antioxidant (TAC, TOS, MDA, and CAT) activity of *A. parviflora* was also determined. Phytochemical screening showed a positive result for the steroids, terpenoids, alkaloids, di- and triterpenoids, phenols, flavonoids, tannins and volatile oils. These constituents have different activities which are helpful in healing ulcer. Flavonoids have free radical sacvenging and antioxidant activity. The important derivative of flavonoids is quercetin. Flavonoid increases mucus production and also have antihistaminic properties which reduces the histamine production and reduction of mast cells which are produced by the aspirin.

The main mechanism of action for the gastroprotective effects of this flavonol are its proton pump inhibitor and antioxidant properties. Oral administration of NSAIDs, such as aspirin, indomethacin have several undesirable effects on the gastrointestinal tract and increase the likelihood of myocardial infarction.

Flavonoids also have anti-inflammatory properties without any ulcerogenic action as a side effect and thus show a great advantage in the treatment of peptic ulcers (Kelly et al., 2009).

Phenolic substances e.g. phenolic acid and tannins contribute directly to antioxidant activity. Tannins are important constituents of *A. parviflora*. These are poly phenols and water soluble compound present in plants. They have astringent properties so they are used primarily in medicine.

Tannins precipitate micro proteins to the peptic ulcer location. Tannins form an impermeable layer over the coating that prevents the gut secretions and protects the basic mucosa from toxins and other irritants. Tannins endorse resistance to the achievement of proteolytic enzymes, a related activity against *Helicobacter pylori*. They also inhibit gastric acid secretion (Wallace et al., 2000).

This study gives the suggestion that in case of gastric ulcer the oxidative stress is increased then the MDA activity and levels of TOS are further increased in ulcerated group, while the levels of TAC and CAT are decreased for the same group. Antioxidants have defensive properties against gastric ulcer and many other ailments (Saravanan, 2011). The oxidative alteration in the cellular membrane or intracellular molecules occurs by the imbalance between ROS and antioxidant defense mechanism. LPO causes loss of membrane fluidity, impaired ion transport and membrane integrity (Tandon et al., 2004).

It has been concluded from the results that when aspirin was given at a dose rate of 20 mg/kg, TOS and MDA were enhanced significantly while TAC and CAT activity were reduced significantly. Aspirin encourages the reactive oxygen metabolites that may play a part in gastric damage. These reactive species cause damage to the biochemical markers e.g. lipid and increased the production of free radicals that increased MDA production and decreased CAT and these free radicals production also because of impairment of cellular enzyme that involve in defensive mechanism of gastric ulcer such as total antioxidant capacity and CAT activity (Filho et al., 2012).

The curative ratio for ulcer of treated groups was computed. *A. parviflora* decreased gastric lesions dose dependently. *A. parviflora* significantly decreased the gastric MDA content while it increased CAT activity compares to aspirin. This showed that the antiulcer activity of the *A. parviflora* might be recognized by these tests.

Conclusion

In the present study, *A. parviflora* seeds extract proved to be gastroprotective. It also significantly enhanced the TAC and CAT activity comparable to synthetic antiulcer drug while it caused a significant reduction in TOS and MDA levels which indicates it as an antioxidant. Overall study revealed that extract of *A. parviflora* at 250, 500, and 750 mg/kg showed gastric protection of 47.5, 58.1, and 73.5%, respectively.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations: NSAIDs, Non-steroidal anti-inflammatory drugs; **HCl,** hydrochloric acid; **HCO_3,** bicarbonate; **PG,** prostaglandin; **COX,** cyclooxygenase; **LPO,** lipid peroxidation; **CAT,** catalase; **SOD,** superoxide dismutase; **GI,** gastrointestinal; **MDA,** malondialdehyde; **TOS,** total oxidant status; **TAC,** total antioxidant capacity.

REFERENCES

Ahmad S, Ali A, Beg H, Dasti AA, Shinwari ZK (2006). Phytochemical screening of aerial parts of *Artemisia parviflora*. Pak. J. Weed Sci. Res. 12:183-190.

Ahuja J, Suresh J, Deep A, Madhuri, Pratyusha R (2011). Phytochemical screening of aerial parts of *Artemisia parviflora*: A medicinal plant. Der. Pharm. Lett. 3:116-124.

Alsabri SG, Rmeli NB, Zetrini AA, Mohamed SB, Meshri MI,Aburas KM, Bensaber SM, Mrema IA, Mosbah AA, Allahresh KA, Hermannand A,Gbaj A (2013). Phytochemical, anti-oxidant, anti-microbial, anti-inflammatory and anti-ulcer properties of *Helianthemum lippii*. J. Pharmacogn. Phytochem. 2:86-97.

Aslam B, Awan T, Javed I, Khaliq T, Khan JA, Raza A (2015). Gastroprotective and antioxidant potential of *Glycyrrhizaglabra*on experimentally induced gastric ulcers in albino mice. Int. J. Curr. Microbiol. Appl. Sci. 4(2):451-460.

Aslam B, Majeed W, Javed I, Muhammad F, Khaliq T, Khan JA, Ali A, Sindhu ZD (2013). Gastroprotective effect of *Berberis vulgaris* (zereshk) seeds against gastric ulcer induced by aspirin in male adult albino mice. Indo. Am. J. Pharm. Res. 3:4518-4527.

Awaad AS, Meligy RME, Soliman GA (2013). Natural products in treatment of ulcerativecolitis and peptic ulcer. J. Saudi Chem. Soci. 17:101-124.

Baigent C, Blackwell L, Collins R (2009). Aspirin in the primary and secondary prevention of vascular disease. Collaborative meta analysis of individual participant data from randomized trials. Lancet 373:1849-1860.

Baumgart DC, Sandborm WJ (2007). Inflammatory bowel disease: Clinical aspects and established and evolving therapies. Lancet 369:1641-1657.

Begum R, Bilal A, Ijaz J, Tanweer K, Faqir M, Ahmad R (2014). Gastro protective and antioxidant effect of *Euphorbia prostrata* against indomethacin induced gastric ulcers in healthy adult male albino rabbits. Int. Res. J. Pharm. 5:846-850.

Erel O (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. 37:277-285.

Erel O (2005). A new automated colorimetric method for measuring total oxidant status. Clin. Biochem. 38:1103-1111.

Filho TP, Olaitan SB, Almedia DA, Lima JC, Ascencio PGM, Ascencio DS (2012). Evaluation of antiulcer activity and mechanism of action of methanol stem bark extract of *Lafoensia pacari* A. St.-Hil. (Lytraceae) in experimental animals. J. Ethnopharmacol. 144:497-405.

Gisbert JP, Pajares JM (2003). *Helicobacter pylori*infection and perforated peptic ulcerprevalence of the infection and role of antimicrobial treatment. Helicobacter 8:159-167.

Goswami M, Kulshreshtha M, Chandana VR, Yadav S (2011). Anti-ulcer potential of *Lawsonia inermis* leaves against gastri ulcer in rats. J. Appl. Pharm. Sci. 1:69-72.

Goth L (1991). A simple method for determination of serum catalase and reversion of reference range. Clin. Chem. Acta. 2:143-51.

Hamid AR, Foong CP, Ahmad Z, Hussain MK (2012). Antinociceptive and anti-ulcerogenic activities of the ethanolic extract of *Annona muricata*leaf. Braz. J. Pharmacogn. 22:630-41.

Herbert M, Jackson C, Ekpo M, Okopedi E, Anah V (2011). Gastroprotective effects of ethanolic leaf extract of *Musa paradisiaca* in rats. J. Chem. Pharm. Res. 3:322-27.

Kelly SLM, Dias GEN, Pinto MEF, Ferreira AL, Brito AMS, Lima CAH, Filho JMB, Batista LM (2009). Flavonoids with gastroprotective activity. Molecules 14:979-1012 .

Maity S, Chaudhri T, Vedasiromoni JR, Ganguly DK (2003). Cytoprotection mediated antiulcer effect of tea root extract. Ind. Institute Chem. Biol. 35:213-219.

Malairajan P, Geethav, Narasimhan S, Veni KJK (2008). Evaluation of antiulcer activity of *Polyalthia longifolia*(sonn). Thwaites in experimental animals. Indian J. Pharmacol. 40:126-138.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxide in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95:351-358.

Saravanan J, Venkatesh P, Soumya V, Hariprasath K, Prasad RH, Kumar KTM (2011). Antiulcer and antioxidant activity of methanolic leaf extract of *Soymida febrifuga*. J. Pharm. Res. 5:528-531.

Steel RGD, Torrie GH, Dickey DA (1997). Principles and procedures of statistics. 3rd Ed. McGraw Hill, New York. pp. 1746-1762.

Tandon R, Khanna HD, Dorababu M, Goel RK (2004). Oxidative stress and antioxidant status in peptic ulcer and gastric carcinoma. Indian J. Physiol. Pharmacol. 48:115-118.

Vogel HG (2002). Drug discovery and evaluation, pharmacological assay. 2nd Ed, Springer, Germany. pp. 759-767.

Wallace JL, McKnight W, Reuter BK, Vergnolle N (2000). NSAID-induced gastric damage in rats, requirement for inhibition of both cyclooxygenase 1 and 2. Gastroenterology 119:706-714.

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