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Full Length Research Paper

Screening of crude extracts of twelve medicinal plants and "wonder-cure" concoction used in Nigeria unorthodox medicine for activity against *Mycobacterium tuberculosis* isolated from tuberculosis patients sputum

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The antimicrobial activity of extracts of twelve Nigerian medicinal plant species and a "wonder cure" concoction (Epa –Ijebu) used in traditional medicine for the treatment of tuberculosis and cough were screened for activity against *Mycobacterium tuberculosis* isolated from tuberculosis patient sputum and the control strains of *M. tuberculosis* (H37RV). Both ethanolic and aqueous solution of the extract of *Allium ascalonicum*, *Terminalia glaucescens*, *Allium cepa* and *Securidaca longepedunculata* (ethanolic extract only) at 0.05 g/ml as well as aqueous solution of "wonder cure" concoction at same concentration inhibited the growth of *M. tuberculosis*. The phytochemical analysis of the plant extract and the Epa-Ijebu showed the presence of bioactive compounds: tannin, flavonoid, alkaloids, phylobatanin, anthocyanin, reducing sugar, saponin and anthraquinone. Our results offer a scientific basis for the traditional use of aqueous and ethanolic extracts of *A. ascalonicum*, *T. glaucescens*, *A. cepa*, *S. longepeducunlata* (ethanolic extract only) and aqueous solution of the "wonder cure" concoction against *M. tuberculosis.* However local herbs such as *Nicotiana tabacum*, *Allium sativum*, *Aframomum melegueta*, *Aprus precatorius*, *Xylopia aethiopica*, *Tetrapleura tetraptera*, *Crinium jagus*, and *Garcinia kola* were ineffective *in vitro*.

Key words: Medicinal plants, unorthodox medicine, Epa-ijebu, *M. tuberculosis,* sensitivity.

INTRODUCTION

Tuberculosis (TB) is an infectious disease, caused by the bacterium called *Mycobacterium tuberculosis*. It was first isolated by Robert Koch in 1882 (Ait- Khaled and Enarson, 2003). At the time, TB was rampant, causing 1/7 of all deaths in Europe and 1/3 of deaths among productive young adults (Prescott et al., 2005). Today TB remains a problem of global importance. Among communicable diseases, TB is the second leading cause of death worldwide killing 2 million people each year (Frieden et al., 2003).

The upsurge of TB cases has been noticed in developing countries (WHO, 2003). In Nigeria, like in most other developing countries, the tuberculosis situation has worsened over the past few years. Several factors have been associated with the TB upsurge which have a distinct difference in symptoms from earlier out-breaks of the disease. These include the current Human Immunodeficiency Virus (HIV) pandemic and increase in cases of drug resistant strains of TB bacilli (Onwujekwe, 2005). A prevalence of 9.2% has been reported in one study in Nigeria and a case of fatality rate of 12% in a second study (Salami and Oluboyo, 2002; Salami and Oluboyo, 2003).

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Table 1. Medicinal plants chosen for antimicrobial activity against *M. tuberculosis*.

Plant species investigated	Family	Plant part
Crinium jagus (Thomps.) Dandy	Amaryllidaceae	Bulb
Allium ascalonicum Linn.	Liliaceace	Leaves
Allium cepa Linn.	Liliaceae	Bulb
Xylopia aethiopica (Dunal) A.Rich.	Annonaceae	Fruit
Aprus precatorius Linn.	Papilionoide- Fabaceae	Whole plant
Allium sativum Linn.	Liliaceae	Fruits
Aframomum melegueta K. Schum	Zingiberaceae	Fruits
Terminalia glaucescens Planch. ex Beith.	Combretaceae	Stem
Tetrapleura tetraptera (Shum. et thom.) Taub.	Mimosoide- Fabaceae	Fruits
Garcinia kola Heckel	Clusiaceae	Fruits
Nicotiana tabacum Linn.	Solanaceae	Whole plant
Securidaca longepedinculata Fres.	Polygalaceae	Stem

Trend from orthodox medical treatment to traditional medicine or a combination of both is observed in a large proportion of the population in Nigeria. This is due either to financial constraints or unavailability of manufactured drugs. Also, resistance to drugs obtained from plants is not common unlike the chemically synthesized drugs, some of which are easily metabolized by many pathogens thereby making the drugs ineffective (Ebara et al., 1990 and Gangadharam et al., 1993). There has also been a wide claim by the traditional healers about the pharmacological efficacy of their preparations and prescriptions. For instance, some traditional attendants in Western Nigeria claimed that they cured tuberculosis ('Iko efe') using some medicinal herbs and a "wonder" cure concoction called "Epa-Ijebu". The ingredients used for the preparation of the later include *Citrus aurantifolia* (lime) juice, *Citrus aurantium* (Orombo igun) and *Aframomum melegueta* (Ataare) fruit. Others are animal parts including snake head (various types ground into powder), whole scorpion (powdered) and poisonous rat (powdered). All the above are mixed together in a large pot and cooked until the materials are reduced by halve and then allowed to cool. The resultant product in form of paste are packed into smaller bottle and sold. The concoction is usually added to pap (a slurry of milled corn prepared in boiled water) and drunk. There is a considerable interest by scientists to identify the potentially valuable therapeutic agents contained in these plants and other remedies in order to establish the basis for their uses in folk medicinal practices. These claims need to be verified through scientific and systematic evaluation. Thus, this present study was designed to scientifically evaluate the efficacies of twelve medicinal plants and the potent wonder cure "Epa-Ijebu" used in the treatment of tuberculosis.

MATERIALS AND METHODS

Plant materials and the wonder cure concoction

The plants used in this study were selected from the list of plants used by local herbalist in the preparation of various medicaments used for curing tuberculosis. Twelve plants and plant parts e.g. roots, stem, bark, leave (Table 1) were purchased from local attendants refered to as 'Elewe Omo' in various markets in Lagos State Nigeria namely Oshodi, Mushin, Epetedo, Ajegunle, and Iyana – Ipaja. They were properly identified at the Department of Botany and Microbiology University of Lagos by comparing with existing voucher samples. The plant parts such as the fruits, leaves, and stem were dried at room temperature and crushed into coarse powder by grinding in a clean mortar with pestle while barks of the plant were dried at 80°C for 2 days and subsequently crushed into coarse powder. Aqueous extracts and ethanol extracts were made by weighing 20 g of powdered plant material into the soxhlet flask for extraction. 150 ml of solvent was used both for alcohol and water. The apparatus was allowed to reflux for 3 h and allowed to cool. The alcohol extract, was collected into clean sterile bottles and labeled accordingly. The alcohol extract was dried in the oven at 25°C while the aqueous extracts were freeze-dried. The plant extracts obtained were pure and ground to powder in clean mortar with pestle. They were collected in sterile universal bottles, labeled accordingly and stored in the refrigerator until required for use. The wonder cure concoction (Epa-Ijebu) was procured in prepared form from herb sellers known as 'Elewe Omo' in Mushin Market, a suburb of Lagos, Nigeria.

Bacterial susceptibility testing

The antibacterial activity of the plant extracts and the "wonder cure" concoction were tested on *M. tuberculosis* using proportion method. The test organisms used in this study were culture isolates and control strain (H37RV) of *M. tuberculosis*. The culture isolates were isolated from the sputum of TB (+) patients using standard methods (Salami and Oluboyo, 2002). The organisms were subcultured six weeks before use. They were confirmed to be Acid Fast Bacilli (AFB) and maintained on Lowenstein-Jesen (LJ) medium at 4ºC.

Each extract and the wonder cure concoction were diluted with sterile distilled water to concentrations of 0.2 µg/ml and 0.05 g/ml. To obtain a concentration of 0.2 µg/ml, 0.01 g of each extract/ concoction was dissolved in 5 ml of sterile water and filtered through membrane filter. The filtrate was added to 45 ml of the media in a conical flask. To obtain a concentration of 0.05 g/ml, 1 g of each extract was dissolved in 5 ml of sterile water and also

filtered. The filtrate was added to 15 ml of the media in a conical flask and mixed properly. 10 ml each were later dispensed into universal container and slanted. Fifty LJ slopes were prepared and labeled in triplicates in accordance to the herb extract or concoction contained. The standard drug (isoniazide) at 0.2 µg/ml was also

Table 2. Critical proportion calculations using isoniazide as the standard at lower concentration of herb extracts (0.2 µg/ml).

Name of extract/ concoction	Solvent	(1) Control 10^{-3}	(2) Herb extract 10^{-5}	B (1÷2)	(3) Control 10^{-5}	(4) Herb extract 10^{-3}	A (3÷4)	Critical proportion B:A%	Sensitivity
Crinum jagus	Water	100	100	1	100	100	1	≥ 1	Resistant
	ethanol	100	100	1	100	100	1	≥ 1	Resistant
Allium cepa	Water	100	100	1	100	50	2	≥ 2	Resistant
	Ethanol	100	100	1	100	40	2.5	≥ 2.5	Resistant
Xylopia aethiopica	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Abrus precatorius	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Allium ascalonicum	Water	100	100	1	100	80	1.25	≥ 1.25	Resistant
	Ethanol	100	100	1	100	50	2	≥ 2	Resistant
Allium Sativum	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Aframomum melegueta	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Terminalia glaucescens	Water	100	50	2	100	20	5	≥ 5	Resistant
	Ethanol	100	80	1.25	100	50	2	≥ 2	Resistant
Tetrapleura tetraptera	Water	100	100	1	100	100	1	≥1	Resistant
	Ethanol	100	100	1	100	100	1	≥1	Resistant
Grarcinig kola	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Nicotina tabacum	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Securidaca longepedunculata	Water	100	100	1	100	50	2	≥ 2	Resistant
	Ethanol	100	100	1	100	50	2	≥ 2	Resistant
Salt	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Epa- Ijebu	Water	100	100	1	100	50	2	≥ 1	Resistant
	Ethanol	100	100	1	100	80	1.25	≥ 1.25	Resistant
Isoniazide	Water	100	0	0	100	0	0	≤ 1	Sensitive

prepared and dispensed into three of the LJ slopes accordingly. They were used as positive control. Slopes without herb extracts were used as control medium while slopes containing solvent of each extracts were used as negative control. The fifty slopes prepared were incubated at 85°C for 45 min, cooled and stored in refrigerator at 4°C until required for use.

Bacterial dilutions (10^{-3} and 10^{-5} mg/ml) were prepared for inoculation. 0.1 ml of the two chosen bacterial dilutions

Table 3. Critical proportion calculations using isoniazide as the standard at higher concentration of herb extracts (0.05 g/ml).

Name of extract/ concoction	Solvent	(1) Control 10^{-3}	(2) Herb extract 10^{-5}	B (1÷2)	(3) Control 10^{-5}	(4) Herb extract 10^{-3}	A (3÷4)	Critical proportion B:A%	Sensitivity
Crinum jagus	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Allium cepa	Water	100	0	0	100	0	0	≥ 1	Resistant
	Ethanol	100	0	0	100	0	0	≥ 1	Resistant
Xylopia aethiopica	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Aprus precatorius	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Allium ascalonicum	Water	100	0	0	100	0	1	≤ 1	Sensitive
	Ethanol	100	0	0	100	0	1	≤ 1	Sensitive
Allium sativum	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Aframomum melegueta	Water	100	50	2	100	40	2.5	≥ 5	Resistant
	Ethanol	100	80	1.25	100	50	2	≥ 2.5	Resistant
Terminalia glaucescens	Water	100	50	2	100	20	5	≥ 10	Resistant
	Ethanol	100	80	1.25	100	50	2	≥ 2.5	Resistant
Tetrapleura tetraptera	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Grarcinig kola	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Nicotina tabacum	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Securidaca longepedunculata	Water	100	50	2	100	40	2.5	≥ 5	Resistant
	Ethanol	100	100	1	100	0	0	≤ 1	Sensitive
Salt	Water	100	100	1	100	100	1	≥ 1	Resistant
	Ethanol	100	100	1	100	100	1	≥ 1	Resistant
Epa- Ijebu	Water	100	100	1	100	50	2	≥ 1	Resistant
Isoniazide	Water	100	0	0	100	0	0	≤ 1	Sensitive

was inoculated into all the labeled LJ slopes. The inoculated slopes were loosely closed with a cap to allow for evaporation and incubated at 37°C for 28 days. The results of sensitivity test for *M. tuberculosis* were read on the 28th and 42nd day of incubation.

Phytochemical screening

The screening methods were carried out using the procedure described by Harbone and Sofowora (Harbone, 1984; Sofowora, 1986). The following active constituents were tested for: alkaloids, tannins, flavonoids, cyanogenic glycosides, anthraquinone, saponins, phylobatinnins, anthrocyanosides (anthrocyanin pigment) and reducing sugar

Table 4. Critical proportion calculations using isoniazide as the standard at higher concentration of herb extracts (0.05 g/ml).

Name of extract/ concoction	Solvent	(1) control 10^{-3}	(2) Herb extract 10^{-5}	B (1÷ 2)	(3) Control 10^{-5}	(4) Herb extract 10^{-3}	A (3÷4)	Critical proportion B:A%	Sensitivity of *M. tub*erculosis (H37RV)
Allium cepa	Water	100	0	0	100	0	0	≤ 1	Sensitive
	Ethanol	100	0	0	100	0	0	≤ 1	Sensitive
Allium ascalonicum	Water	100	0	0	100	0	0	≤1	Sensitive
	Ethanol	100	0	0	100	0	0	≤1	Sensitive
Terminalia glaucescens	Water	100	100	1	100	50	2	≥2	Resistant
	Ethanol	100	100	0	100	0	0	≤1	Sensitive
Securidaca longepedunculata	Water	100	0	0	100	0	0	≤1	Sensitive
	Ethanol	100	0	0	100	0	0	≤1	Sensitive
Epa- Ijebu	Water	100	100	0	100	100	0	≤1	*Sensitive*
Isoniazide	Water	100	100	0	100	100	0	≤1	Sensitive

compounds.

RESULTS AND DISCUSSION

The results on Table 2 showed that at lower concen-tration (0.2 µg/ml) critical proportion level of isoniazide, *M. tuberculosis* was resistant to all the plant extracts and the "Epa-Ijebu". At concentration of 0.05 g/ml, extracts obtained from *A. cepa*, *A. ascalonicum*, *T. glaucescens*, *S. longepedunculata* as well as the wonder cure concoct-tion inhibited the growth of *M. tuber-culosis* as shown in Table 3. All the plant extracts that inhibited the growth of the culture isolates of *M. tuberculosis* also inhibited the growth of the control strains (H 37 RV) of *M. tuberculosis* H37RV (Table 4).The results also showed that *M. tuberculosis* was sensitive to the ethanolic extract of *S. longepedunculata* but was resistant to its aqueous extracts. All the tested plants showed to have tannins, saponin, alkaloids, and anthraqui-none but none posses cyanogenic glycosides and anthocyanin pigment (Table 5).

This present study revealed that four of the plant extracts (*A. cepa*, *A. ascalonicum*, *T. glauce-scens*, *S. longepedunculata)* as well as the wonder cure concoction showed activity on both the test organism and the control strain. Adjanohoun et al. (1991) and Adeleye and Opia (2003) had earlier reported the efficacy of *Allium cepa* and *Allium ascalonicum* as cough remedies. Also Akinyemi et al (2005) reported the antimicrobial property of *T. avicenoides* onmethi-cillin resistant *Staphylococcus aureus.* The activity of the wonder cure concoction (Epa – Ijebu) may be due to its acid nature (it has a low pH).

The inactivity of the remedies at lower concentration of 0.2 µg/ml (critical proportion level of isoniazide) may be due to the fact that the active substances responsible for inhibition of *M. tuberculosis* were in crude form when compared to the standard drug isoniazide. It is worthy of note that eight other plant extract screened including *Nicotiana tabacum* did not show activity against *M. tuberculosis.* Previous study has shown that *N. tabacum* has no antibacterial effect on organism causing upper respiratory tract infections. Although the traditional use of *Crimium jagus*, *Xylopia aethiopica*, *Aprus precatorius*, *A. sativum*, *A. melegueta* and *Tetrapteura tetraptera* as cough remedy had been well documented (Adjanohoun et al., 1991). Our study shows that they did not inhibit *M. tuberculosis in vitro*.

The phytochemical analysis of the plant extracts showed the presence of biologically active constituents such as alkaloid, tannins, flavonoids, anthraquinones, saponins, plylobatinnis and reducing sugar compounds but none posses cyanogenic glycosides and anthrocyanin pigment. Elsewhere in Democratic Republic of Congo similar observations have been made in plants employed for traditional medicines, which were known to contain the above mentioned bioactive components (Otshudi et al., 2000).

Our findings may provide the rational for the traditional use of both water and ethanol extracts of *A. ascalonoicum*, *T. glaucescens*, *A. cepa* and *S. longepedunculata* as well as aqueous solution of wonder cure concoction "Epa – Ijebu" for therapeutic cure of tuberculosis. The antimicrobial activities could be enhanced if the active compo-

Table 5. Phytochemical analysis of plant extracts and Epa-Ijebu.

Plant species	Tannin	Flavonoid	Saponin	Phylobatanin	Anthraquinone	Cyanogenic glycosides	Alkaloids (3÷4)	Anthrocyanin pigment	Reducing sugar
Crinum jagus	+	+	+	-	+	-	+	-	+
Allium cepa	+	+	+	-	+	-	+	-	+
Xylopia aethiopica	+	+	+	-	+	-	+	-	+
Aprus precatorius	+	+	+	+	+	-	+	-	+
Allium ascalonicum	+	+	+	+	+	-	+	-	+
Allium Sativum	+	+	+	-	+	-	+	-	+
Aframomum melegueta	+	+	+	-	+	-	+	-	+
Terminalia glaucescens	+	+	+	+	+	-	+	-	+
Tetrapleura Tetraptera	+	+	+	-	+	-	+	-	+
Grarcinig kola	+	+	+	-	+	-	+	-	+
Nicotina tabacum	+	+	+	-	+	-	+	-	+
*Securidaca longepedunculat*a	+	+	+	+	+	-	+	-	+
Epa- Ijebu	+	+	+	+	+	-	+	-	+

+ = Presence of active constituents; - = absence of active constituents

nents are purified and adequate dosage determined for proper administration.

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