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ANALYSIS OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *TERMINALIA BROWNII* UPON *ESCHERICHIA COLI & CANDIDA ALBICANS*

[1]Asmait Weldegergis, [1]Senait Medhanie, [1]Biniam Yamane, [2]Mehari Andebrhan,*[1]Kamal C. Semwal & [3]Gangwar, S. K.
[1]Department of Biology, College of Science, Eritrea Institute of Technology, P. Box 12676, Mai Nafhi, Asmara, Eritrea (East Africa)
[2]Department of Chemistry, College of Science, Eritrea Institute of Technology, P. Box 12676, Mai Nafhi, Asmara, Eritrea (East Africa)
[3]APRI, R.P.C.A.U., Pusa, Samastipur, Bihar, India
*Corresponding author email: kamalsemwal@gmail.com

ABSTRACT
Terminalia brownii is one of the plant species found in Eritrea, primary used for medicine in areas where traditional medication is most practiced. Hence the purpose of the present study was to investigate the anti-microbial effects of *T. brownii* Fr., extraction (stem bark and whole roots) against the bacteria *Escherichia coli* and the fungi *Candida albicans* with qualitative analysis of the phytochemical constituents of the plant. Consequently the study showed that phytochemicals found in the stem bark and root extracts of *T. brownii* indicates their potential to be used as a source and may supply as novel medicines with wide spectrum of antibacterial and antifungal activities, which provides a support to some traditional uses of the medicinal plant. The variation in zone of inhibition in petri-dishes was due to the different degree of efficacy and difference in phytoconstituents.

KEYWORDS: Traditional medicine, anti-microbial, activity,

INTRODUCTION

Microbial infections pose a health problem throughout the world and plants are a possible source of antimicrobial agents (Kareru *et al.*, 2008). Plants have long history of usage as therapeutic agents and the main source of medicines prior to the advances of modern medicine (Cock, 2015). Over 9000 plants have been known for medicinal application in various cultures and countries (Arun *et al.*, 2013). Natural drugs from the plants are gaining popularity because of several advantages such as often having fewer side-effects, better patient tolerance, being relatively less expensive and acceptable due to a long history of use (Nahiba and Hamdani, 2014). Traditionally used medicinal plants produce a variety of compounds for the treatment of various ailments (Abraham *et al.*, 2016; Biniam *et al*., 2017). The World Health Organization defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (Kaliyaperumal *et al*., 2013). Approximately 60%- 80% of the people in Africa rely on traditional remedies to treat themselves for various diseases (WHO, 2003). Eritrean society has a long history of practicing traditional or herbal medicine that also has links to cultural values and beliefs. It survives in the form of home remedies, religious or spiritual healing, traditional herbal medicine and other rituals of the traditional healer. It survives because the traditional healer has gained the trust and confidence of the people and is still the most accessible health care provider for many people and communities (Biniam *et al.*, 2016). However, the use of traditional medicine for the treatment of diseases has its own advantages and disadvantages. Of the advantages, the major one is that it is a potential source of new drugs (Abayneh, 2005) and has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of resource poor communities and the local therapy is the only means of medical treatment for such communities (Haile and Delenasawa, 2007). Its major disadvantage is that most preparations expected to have medicinal value are not evaluated scientifically for their efficacy and safety. Traditional medicines are not standardized nor dispensed to patients in scientific doses or strictly regulated quantities (Abayneh, 2005). The WHO notes, however, that "inappropriate use of traditional medicines or practices can have negative or dangerous effects" and that "further research is needed to ascertain the efficacy and safety" of several of the practices and medicinal plants used by traditional medicine systems (Berhane *et al*., 2014). Phytochemicals are naturally occurring in the medicinal plants, leaves, and roots that have defense mechanism and protect from various diseases (Haile *et al.*, 2015). Primary metabolites are chemical substances aimed at growth and development. Secondary metabolites are a source of new antimicrobial products and inexpensive starting materials for synthesis of many known medicine, insecticide, fungicide and drugs etc, (Enass, 2003) such as terpenoid, alkaloids and phenolic compounds. The complete phytochemical investigations of medicinal plants should be carried out, because these secondary metabolites are responsible for medicinal activity by eliciting pharmacological and toxicological effects in human and animals (Azmir *et al.*, 2013). *Terminalia* species, distributed in the tropics and sub-tropic regions, are famous for their usefulness in traditional medicine. *Terminalia brownii* Fr., from family Combretaceae, which

belongs to genus *Terminalia* occurs in parts of Eastern and Central Africa (Francis *et al*., 2013). In Eritrea and Ethiopia it is known as *Weiba* in Tigrigna language (Orwa *et al*., 2009). Deciduous shrub or small tree up to 15m tall, often low-branching; bark surface fissured, grey, inner bark thick, fibrous, dull red-brown; crown umbrella-shaped, with spreading branches; twigs initially hairy, becoming glabrous. Leaves alternate – spirally arranged at the end of the branches; simple, entire, elliptic–obovate, 5-8cm long, 3-5cm wide, petiole 2-3cm, glabrous below, apex pointed. Flowers small, white in 7-10cm long many-flowered spikes, with an unpleasant smell. Individual flowers consist of calyx, 5 stamen and ovary; no petals. Flowers are hermaphroditic or male. The fruit is a woody samara. It is broadly elliptic–ovate, 2½-3½cm long, flat, with a 1-1½cm broad wing surrounding the central fruit part. The fruit is purple red at maturity, turning chocolate brown with age (Eduards, *et al*., 1995 and Mosango , 2013).

Traditionally, in Eritrea it is used to treat bacterial, fungal and viral infections diarrhea, cut wounds, gonorrhea, cough, jaundice, hepatitis, liver cirrhosis, and yellow fever. Roots are used against allergic reactions. In veterinary medicine, leaves are given to livestock to treat diarrhoea and leaf extracts are applied for the treatment of conjunctivitis (Biniam *et al.*, 2017). Pastoral communities in Sudan, Kenya, Uganda and Tanzania use leaf and bark decoctions to treat worms and babesiosis in cattle and goats (Mosango, 2013).

The aim of this study was to investigate the anti-microbial effects of *T. brownii* extraction against *E. coli* and *C. albicans* and qualitatively screening of the phytochemical constituents of the extracts using petroleum ether, ethyl acetate, ethanol and aqueous extractions. Furthermore, the anti-bacterial and anti-fungal activity of the plant by performing a sensitivity analysis and to recommend the results to the pharmaceutical industries to develop drugs using the phytochemical components which are extracted from *T. brownii.*

MATERIALS & METHODS

Sample collection

The plant in this study was collected from sub zone logo Anseba in Gash Barka, Eritrea. The plant sample was identified and authenticated in the Department of Biology Herbarium in EIT. The antibacterial effect of the plant was evaluated on *E. coli* (ATTC 25922), while the antifungal effect was evaluated using *Candida albicans* (ATCC 2091). All strains were obtained from the National Referral Laboratory, Ministry of Health**.**

Plant extraction Techniques

The stem bark and whole root of the plant were dried under shade at room temperature before it was grinded. The powder was then kept in small plastic bags prior to analysis. Then 100g of the dried powder was extracted with 400mL of petroleum ether, ethyl acetate, ethanol and water by sequential extraction for 48-72 hours at room temperature. The extracts obtained were filtered with Whatmann No. 1 filter paper and dried prior to their extraction by the next solvent. The extracts were then stored until further use.

Preparation of media, Sample extract and Inoculums

Each of 38g Muller Hilton agar (OXOID) and sabouraud agar was mixed with 1L of distilled water and then was sterilized in autoclave at 121°C and 151 Barr pressure for 15 minutes. The sterilized media were allowed to cool to a temperature of about 50°C and poured into petri-dishes with the thickness of the media about 4mm inside the safety cabinet. The solidified plates were kept in the refrigerator of about 2-8°C until further use, 2g powder of each sample was dissolved in 10mL of petroleum ether, ethyl acetate, ethanol and distilled water in test tubes to obtain concentration of 200mg powder/mL. The extraction was done at room temperature for 24 hours. It was then filtered. The extract obtained was then stored in sterile reagent bottles and refrigerated at 4°C until further use. To obtain serial dilutions, different concentrations were prepared from the stock concentration (200mg powder/mL) which was diluted to concentrations of 100mg powder/mL, 50mg powder/mL and 25mg powder/mL. The test organisms were inoculated in peptone water and incubated for 3 hours at 35°C.Turbidity of the suspension was adjusted to match 0.5 McFarland and used for antibacterial sensitivity assay.

A

B

C

D

E

FIGURE 1: **A-** Leaves of *Terminallia brownii*; photo by (Orwa *et al*., 2009); **B-** root sample air dried under shade, **C**- powdered sample; **D**- Stem bark sample air dried under shade; **E-** cold extract using Flocummeter

Antimicrobial sensitivity test

The total activity of *T. brownii* crude extract was carried out by agar-well diffusion method to assess the antimicrobial activity of the prepared extracts. 20mL of aliquots of the inoculated sabouraud dextrose and Muller Hinton agar were distributed into sterile petri-dishes. The agar was left to set and in each of these plates 3 wells (5mm in diameter) were cut using borer tool 5mm in diameter. Alternative wells were filled with 100µL from the different diluted extracts (petroleum ether, ethanol, water and ethyl acetate extracts) using micropipette. Three replicates for each extracts of the tested microbes were made each test. Three different concentrations of the plant extracts were used: 100mg powder in 1mL solvent, 50mg powder in 1mL solvent and 25mg powder in 1mL solvent. The extracts were allowed to diffuse at room temperature for two hours. The plates were then incubated at 37ºC for 24 hours. After incubation the diameters of the resultant growth inhibition zones were measured, average was taken and the mean values were tabulated. The solvents used for extraction and was used as negative controls by adding them to the media instead of the extracts in another set of experiment to confirm that they have got no effect on the growth of the microbes and positive control drugs Clotrimazole for *C. albicans* and gentamicin for *E. coli*.

Qualitative phytochemical analysis

The plant samples were qualitatively analyzed for the presence of different phytochemicals including sterols, saponins, glycosides, alkaloids, tannins, flavonoids, terpenoids and steroids using the standard qualitative methods such as Mayer's test, Keller-Killani test, Salkowski test, saponification test, Liebermann–Burchard test and Fehling's test (Ahuja *et al*., 2011).

1. **Test for Flavonoids**: Lead acetate test: To the extract, a few drops of aqueous basic lead acetate solution were added. Formation of yellow precipitate indicates presence of flavonoids.
2. **Test for Alkaloids (Mayer's test**): To determine the presence of alkaloids few drops of 1.5 v/v HCl and Mayer's reagent was added to 1mL of the extract. The appearance of a turbid solution confirms the presence of alkaloids.
3. **Test for Glycosides:** Kellar-Kiliani test: The test extract was dissolved in glacial acetic acid and after cooling, 2 drops of ferric chloride solution was added. These contents were transferred to test tube containing 2mL of sulphuric acid. A reddish brown color ring formed at the junction of two layers confirms the presence of glycosides.
4. **Test for Tannins**: A few drops of aqueous basic lead acetate solution were added to the extract. Reddish brown bulky precipitate indicates presence of tannins.
5. **Test for Steroids (Liebermann–Burchard test):** 1mL of extract was mixed well with 1mL of chloroform. About 2-3mL of acetic anhydride was added to the above mixture. Two drops of conc. H_2SO_4 was added from the side walls of test tube. Then blue green or dark green color is observed to confirm the presence of steroids.
6. **Test for Saponins**: Presence of saponins was identified by saponification test as follows. To 1mL of extract 3mL of water was added and shaken vigorously. The formation of persistent foam confirms the presence of saponins (Ahuja *et al*., 2011)
7. **Test for Terpenoids**: (Salkowki's test): 1mL of chloroform was added to 2mL of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicates the presence of terpenoid (Wadood *et al.*, 2013).
8. **Sterols:** About 40 mg of the crude extract was dissolved in 2mL of acetic acid. To this solution, 4 mL of concentrated sulfuric acid was added from the side of the test tube without disturbing the solution. A green upper layer indicates the presence of sterol (Joshi *et al.*, 2013).

RESULTS

Phytochemical analyses

The presence of phytochemical compounds such as sterols, saponins, glycosides, alkaloids, tannins, flavonoids, terpenoids and sterol was determined by the change of color or by the presence or absence of a precipitate in different extracts *i.e*. petroleum ether, ethyl acetate, ethanol and distilled water. The results are presented in the following table.

TABLE 1. Qualitative phytochemical evaluation of four different extracts *T. brownii* Note: '+' present; '++' present at high concentration; '-' absence

Phytochemical	Solvents of extract							
	Petroleum ether		E. acetate		Water		Ethanol	
	Stem bark	Root	Stem bark	Root	Stem bark	Root	Stem bark	Root
Flavonoid	-	-	++	++	+	+	+	++
Alkaloid	-	-	-	-	++	++	-	+
Terpenoid	+	+	+	+	+	++	+	++
Tannin	-	-	++	++	+	++	+	-
Glycoside	+	+	+	++	+	+	+	+
Sterol	-	-	+	+	+	+	+	+
Steriod	+	+	+	++	++	++	++	+
Saponin	-	-	+	++	+	+	+	+

Antibacterial and antifungal activity assay

The extracts were prepared using four different solvents and tested at three concentrations against *E. coli* and *C. albicans*. The plant *T. brownii* showed significant activity with zone of inhibition ranging from 6mm to 13mm. The triplicate results of the effect of different extracts of test plant at concentrations of100mg powder/mL, 50mg powder/mL and 25mg powder/mL on test bacterial and fungal are shown in Table 2.

TABLE 2. Triplicate results of zone of inhibition

Name of organism	Type of extract	Conc. In mg powder/ml	Zone of inhibition (mm in diameter)				
			Stand	Petroleum ether	Ethyl acetate	Ethanol	Water
E. coli	Stem bark	100	12	-	-	-	-
		50	12	-	-	-	-
		25	12	-	-	-	-
	Root	100	12	-	7	6	6
		50	12	-	6	6	6
		25	12	-	6	6	6
C. albicans	Stem bark	100	14	-	12	10	11
		50	14	-	10	9	11
		25	14	-	9	8	9
	Root	100	14	-	13	11	12
		50	14	-	11	10	11
		25	14	-	9.5	9	10

DISCUSSION

Medicinal properties of plants unique to particular plant species or groups are consistent with the concept that combination of secondary products in a particular plant is taxonomically distinct (Shemsu *et al*., 2013). For example, Saponins have been ascribed a number of pharmacological action. The important ones being permeabilizing of the cell membrane flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, lipase and kinase enzyme activities (Haile *et al*., 2015). As reported in table 1, the phytochemical studies reported the presence of phytoconstituents like sterols, terpenoids, flavonoids, saponins, alkaloids, tannins, glycosides. All the stem bark and root extracts, specially the ethyl acetate, ethanol and aqueous extracts showed rich content of flavonoids, glycosides and terpenoids. The petroleum extract of the stem bark was found to be rich in glycosides, terpenoids and steroids but the extract showed absence of flavonid tannin and Alkaliod phytochemicals. The aqueous extract of both root and stem bark were found to constitute most of the phytochemical compounds listed above. The result suggests that the aqueous, ethanol and ethyl acetate extracts are efficient solvents for extraction of phyto-constituents from the root and stem bark of *T. brownii*. So this medicinal plant holds promises as source of pharmaceutically important phytochemicals (Table- 2) summarizes the microbial growth inhibition of the plant extracts of four solvents at three different concentrations. The results showed inhibition diameters ranging from 6mm to 13mm and the inhibition zones were found to be decreasing as the concentrations of the extracts were reduced from 100mg powder/mL to 50mg powder/mL and then to 25mg powder/mL. The maximum antimicrobial activity was shown by *C. albicans*, while minimum antimicrobial activity was exhibited by *E. coli*. In this investigation the root extract exhibited excellent antibacterial and antifungal activities against all the tested microbes when compared with stem bark extract. Petroleum ether extracts of root and stem bark, which had exhibited the lowest phytochemical constitution, showed lowest antimicrobial against all the test organisms. The reason can possibly be because the Petroleum ether extract of both stem bark and root constitute poor phytochemical compounds qualitatively.

In this in-vitro study, *T. browii* at concentrations of 100mg powder/mL, 50mg powder/mL and 25mg powder/mL showed significant antifungal activity against *C. albicans* when compared with standard but less antibacterial activity against *E. coli*.

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