PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *DOVYALIS ABYSSINICA*

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Abstract

The traditionally use of plant materials for the treatment of various diseases is very common in Ethiopia. *Dovyalis abyssinica* is one of these plants. This plant is native to Africa. The aim of this study was to determine the phytochemical composition and antibacterial activity of methanol extract of leaves of *Dovyalis abyssinica*. The methanol extract was prepared by maceration and the fractions were obtained by successive fractionation of the methanol extract with n-hexane, dichloromethane, n-butanol and followed by distilled water. The crude methanol leaves extract revealed the presence of phytochemical screening such as flavonoids, alkaloids, saponins, tannins, phenolic, anthraquinones, quinones and terpenoids. Crude methanol and fractionates extract of the leaves were screened for anti bacterial activity against in gram positive bacterial (*Staphylococcus aurous* and *Enterococcus faecalis*) and gram negative (*Escherichia coli*) species using agar well diffusion method at concentrations of 500, 1000 and 10,000 µg/mL. Crude methanol extract exhibited greater activity against gram positive bacteria while n-butanol fraction revealed greater activity against gram negative bacteria. Antibacterial effect of plant extracts might due to the presence of concentrated bioactive compounds or due to synergistic activity of two or more active metabolites. This finding provides a scientific evidence for claimed traditional use of *Dovyalis abyssinica*.

Key words: Phytochemical, Antibacterial activity, *Dovyails abyssinica,* Agar well diffusion, *Escherichia coli, Staphylococcus aurous, Enterococcus faecalis*

Introduction

Medicinal plants are an important source for the therapeutic remedies of various ailments[1]. In developing countries, medicinal plants are mainly used for the treatment of different disease related to microbial infections[2].

Traditional medicine is defined by the World Health Organization (WHO) can be summarized as the sum total of all the knowledge, beliefs and practices that are used in diagnosis, prevention and elimination of physical, mental or social imbalance[3]. According to a report of World Health Organization, most world's populations depend on traditional medicine for their primary health care needs[4].

Ethiopia is a tropical country with varied ecological and climatic conditions that making the country home to diverse plants[5]. Most of Ethiopian people use traditional medicine for the treatment of infectious disease[6] and due to high cost of modern drugs, paucity and inaccessibility of modern health services, and cultural acceptability of

traditional medicine[7]. Some medicinal plants are available in local markets in Ethiopia[8]. Among there *Dovyails abyssinica* are wound healing.

Dovyalis abyssinica (A. Rich), commonly called African gooseberry which is native to Africa[9] and belongs to the small genus *Flacourtiaceae*[10, 11, 12]. Geographically the plant is distributed in Ethiopia, Eritrea, Somalia, North Kenya, Tanzania and Malawi. In Ethiopia, it is usually found along river courses in humid lower highland forest and Wet Weyna Dega agrodimatic zones. The plant is commonly known as *Koshim* (Amharic), *Ankakute* (Oromifaa), *Ongolatz* (Sidama) and *Aihada* (Tigrigna) [13].

Dovyalis abyssinica **(Figure 1)** is a spiny evergreen shrub or tree, up to 5m height, with a rounded crown. The bark is ash grey, almost always supporting lichens. The branches are armed with stout spines, up to 1½ cm long. The branch lets are covered with numerous dotted pores (lenticels). Leaves are oval to obviate, up to 5-7 cm long and 3 cm wide with a rounded tip, edges unevenly rounded. It is shiny, dark green, with reddish stalks and veins[11,12].

Figure 1. Photograph of *Dovyails abyssinica* plant (Photo by: Biruk Bezabeh Yimam)

The *Dovyalis abyssinica* plant parts are used for the treatment of different illness such as; - leaves are used traditionally to treat gonorrhea, brucellosis, teeth problems, [14]tapeworm, sore throat, [15] headache and diarrhea [16] in humans and mastitis in animals [14]. The roots are used traditionally utilized to treat gonorrhea, bilharzias, stomach-ache, fever[11] typhoid, diarrhea [17]colic pain in infants and headache[18]. The stems are used traditionally for maintaining oral hygiene and toothbrush sticks [10]. Fruit is eaten to treat abdominal pain and cancer [8, 19]. In addition to its use as a source of food and for the treatment of the above mentioned ailments, personal communications with some local traditional

practitioners has revealed that the fruit, leaf, stem and root of *Dovyalis abyssinica* are used for wound healing purposes.

Dovyails abyssinica plant has shown antibacterial and antifungal activity based on different concentration that explained in different literature. The antimicrobial activity leave of *Dovyails abyssinica* by using 80% methanol were detected against the bacterial strains of *Bacillus cereus, Neisseria gonorrhea, Shigella flexineri, Staphylococcus aureus* and on the fungal strain of *Aspergillus flavas, Aspergillus niger, Candida albicans, Trichophyton mentagrophytes, Cryptococcus neoformas* and *Trichophyton violacum*[15]. The antimicrobial activity methanol extract of root *Dovyails abyssinica* were inhibited the growth of *Salmonella typhi, Shigella dysenteriae, Klebsiella pneumonia, Candida albicans, Escherichia coli*[17] and *Staphylococcus aureus*[20]. In addition, antimicrobial activity fruit of *Dovyails abyssinica* was active against *Staphylococcus aureus* and *Trichophytonrubrum*[12]. Also, the antimicrobial activity of the stem of *Dovyails abyssinica* extracted by using methanol, aqueous and dichloromethane showed inhabitation against, *Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumonia*[10]. According to the above mentioned the root, leave, stem and fruit shows antimicrobial activity[10, 12,15,17,20].

There is no report on composition of crude and fractionation of the crude against selective pathogenic bacteria. The present study was conducted to investigate the phytochemical composition and antibacterial activity of methanol extract leave of *Dovyails abyssinica*.

Materials and Methods

Chemicals

Methanol (99.9%), wagner's reagent, sulphuric acid, n-butanol, n-hexane, dimethyl sulfoxide (DMSO) and sodium bi carbonates (Samir Tech-Chem Pvt.Ltd.,India), ferric chloride, benzene, glacial acetic acid, ammonia, chloroform and dichloromethane (Lobachemie Pvt.Ltd.,India), potassium hydroxide, anhydrous calcium sulphate and sodium hydroxide (central drug house Pvt.Ltd.,India), sodium chloride and Mueller-Hinton Agar (Hi Media Laboratories Pvt. Ltd., India) . All the chemicals and solvents were of analytical grade.

Apparatus

Drying oven (B-13,okhia phase-2,New Delhi-20, India), refrigerator (RT34SUMG,Thailand), water bath (Re201BL,Model India), electronic balance (1810-BA Model, China), rotary evaporator attached with rotary vacuum (Model,YC7124,China), incubator (DHP-9052B Model), desecrater, cork borer, water distiller, autoclaved and McFarland density meter (DEN-18 Model).

Collection of plant material

The fresh leave of *Dovyalis abyssinica* was collected from Dessie, Amhara regional state which is 401 Km North of Addis Ababa. It was located at a latitude and longitude of 11°8′N39°38′E, with an elevation between 2,470 and 2,550 meters above sea level.

Preparation of the plant material

After collection, the leaves were rapid washed with tap water to remove dirt or dust and dried under shade in chemistry laboratory within the school of chemistry. The leaves were ground to coarse powder using laboratory pestle and mortar. The powder sample was weighed and stored in air tight containers until extraction commenced[14].

Extraction

The crude methanol extract of leaves of *Dovyails abyssinica* was prepared by the maceration method according to previously reported literature [15,21]. Powdered leave material (200.0 g) was soaked in a clean flask containing methanol in 1000.0 mL for three days with frequent manual shaking. The resulting extract was filtered with filter paper (whatmann filter paper No 1) and the supernatant was concentrated under reduced pressure at 40 ^{0}C by using rotary evaporator. The dried extract was transferred into vials and kept in the refrigerator at 4 ^{0}C until further use[10, 17].

Fractionation of the crude extract

Fractionation of crude extract was performed using solvents of different polarity (n-hexane, dichloromethane and n-butanol). The solvent fractionation was done by liquid-liquid extraction of a suspension of 20.0 g of crude methanol extract and 100.0 mL of distilled water with the above mentioned solvents. Finally, the fractions were dried with anhydrous calcium sulphate and concentrated by using rotary evaporate. Each fractionation was repeated three times. The phytochemical screening and antibacterial activity test were conducted for each of the fractions [22,23].

Phytochemical Screening

The crude extract and solvent fractions were subjected to the following preliminary phytochemical studies.

Test for alkaloids

The extract (1.0 mL) was treated with Wagner's reagent (1.27 g iodine and 5.0 g of potassium iodide was dissolved in 100.0 mL distilled water). The formation of a reddish brown precipitate was indicates the presence of alkaloids[24].

Test for flavonoids

The extract (1.0 mL) was transferred into test tube and treated with four drops of sodium hydroxide solution. Formation of a yellow colour was indicates the presence of flavonoids[25].

Test for saponins

The extract (2.0 mL) was transferred into test tube. Four drops of sodium bicarbonates solution was added. The test tube was shaken vigorously and left for 3 mins. Formation of honeycomb like froth was indicates the presence of saponins[26].

Test for tannins

The extract (1.0 mL) was transferred into test tube. Four drops of 5 ferric chloride solution was added. Formation of a greenish black precipitate was indicates the presence of tannins[27].

Tests for Steroids

The extract (1.0 mL) was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red color indicated the presence of Steroids[26].

Test for phenolic compounds

The extract (1.0 mL) was diluted to 3.0 mL of distilled water and filtered. To this, four drops of 5 ferric chloride solution were added. Formation of a dark green colour were indicated the presence of phenolics [28].

Test for anthraquinones

The extract (1.0 mL) was shaken well with 10.0 mL benzene and filtered. Then 0.5 mL of ammonia solution was added to the filtrate and stirred. Formation of a Violet colour were indicated the presence of anthraquinones [25].

Test for Quinones

The extract (1.0 mL) was transferred into test tube. 1.0 mL of Conc. sulphuric acid was added. Formation of red colour was indicated the presence of quinines [29].

Test for Terpenoids

The extract (1.0 mL) with 2.0 mL of chloroform was added. Then 3.0 mL of conc. sulphuric acid were added carefully to form a layer. Formation a reddish brown colour was indicated the presence of terpenoids[27].

Test for cardiac glycosides

The extract (0.5g) was dissolved in 2.0 mL of glacial acetic acid containing four drops of ferric chloride. Then 2.0 mL of conc. sulphuric acid was added under layered. Brown ring was formed at interphase indicated the presence of deoxy sugar which was the characteristic of cardiac glycoside[25].

Invitro antibacterial studies

Bacterial test organisms and standard antibacterial disc

The standard American Type Cell Culture (ATCC) bacterial species of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Enterococcus faecalis* were obtained from Amhara national regional state health bureu Dessie regional health research laboratory. The standard antibacterial disc used for the study was ciprofloxacin 5 µg/well (positive control).

Media preparation

Muller-Hinton agar powder (19.0 g) was suspended in 500.0 mL of distilled water in a flat-bottomed conical flask. The mixture was heated with frequent agitation and boiled for one minute to completely dissolve the media. The flask was then tightly closed using cotton wool and further covered with aluminum foil. The mixture was autoclaved for 15 minutes at 121 after which it was left to cool down to room temperature. The media was poured in the Petri dishes in a laminar flow to give uniform depth of 4 millimeters. The Petri dishes containing the media were then placed in sterile plastic bags and stored at a temperature of 6 before use[30].

Antibacterial activity assay

The antibacterial activities of the extracts were tested using agar well diffusion method. The sterile Mueller Hinton Agar (MHA) media was prepared[31]. The bacterial test organisms like *Escherichia coli, Staphylococcus aureus* and *Enterococcus faecalis* were spread over the agar well plates by using separate sterile cotton buds[32]. Then, an equal distance hole with a diameter of 6.0 mm was punched aseptically using sterile cork borer tip. The extracts (0.5 g) were dissolved in1.0 mL DMSO to get 500 mg/mL stock solution. Different concentrations of the extracts (500 – 10,000

µg/mL) were prepared by dilution of the stock solution in DMSO and poured into the well using micropipette. The next steps were the addition of ciprofloxacin 5 µg/well was placed as positive control disc and DMSO (90.0 µl/well) was placed as negative control disc. The plates were incubated at 37 ^{0}C for 24 h. The extracts were tested in triplicates and the diameter for the zone of inhibition was measured as millimeter (mm) and the results were expressed as mean ± standard deviation.

Results and Discussion

Phytochemical screening result

Evaluation of the preliminary phytochemical screening of the methanol extract of the leaves of *Dovyails abyssinica* plant revealed the presence of flavonoids, alkaloids, saponins, tannins, phenol compound, anthraquinones, quinones and terpenoids. The result is in line with the findings of Geyid et al.[15], who reported phenol compounds, tannins, terpenoids and saponins were present and cardic glycosides was absent by using 80 methanol .On the contrary, the phytochemical screening results differ from the report by Geyid et al.[15], who reported Alkaloids were absent. Differences in results might be related to the geographical distribution of the plant and percentage of solvents used[33].

Tannins and alkaloid were detected in both methanol extract and each solvent fraction. While, Steroids and cardiac glycosides were absent in each extracts.Moreover, dichloromethane and n-hexane fraction alkaloids, tannins and quinones in common. Amongst all fractions, the n-butanol fraction appeared to be relatively rich in secondary metabolites as shown from show in Table 1.

Antibacterial susceptibility assay

In this investigation, the antibacterial activities of both crude methanol extract and each solvent fraction were evaluated using agar well diffusion method at concentration of 500, 1000 and 10,000 µg/mL as shown in Table 2,3and 4. Among the test bacteria, the maximum average zone of inhibitions at 10,000 µg/mL concentration in gram positive bacterial species were determined to be 12.0±0.8 mm *E.faecalis* and 14.0±0.8 mm for *S. aureus*. On the other hand, the maximum average inhibitions, at similar concentration in gram negative bacteria species was 11.0 ±0.8 mm for *E.coil*. In contrary, no zone of inhibition was observed in 500µg/mL of dichloromethane extract against *E.coil*.

Furthermore, zone of inhibition of the n-butanol fraction at 10,000µg/mL concentration was greater than that of crude methanol extract at equal concentration, with against *E.coil*. While, similar a greater value of inhibition was observed in dichloromethane fraction than the crude methanol extract at equal concentration (10,000µg/mL), with against *E.coil.* In the opposite, the zone of inhibition of the crude extract was greater than that of the n-hexane fraction at equal concentrations against *E.coil* (Table 4).

The crude methanol extract show greater antibacterial activity against gram positive bacterial test organisms (*S. aureus* and *E.faecalis*).The result is in agreement with the findings of Geyid et al.[15], who reported *S. aureus* was inhibit by using 80 methanol. On the other side, the present finding differs from that of Geyid et al.[15], who reported *E. coil* was not detected by using 80 methanol. Differences in results might be related to the geographical distribution of the plant and percentage of solvents used[33].

The plant extract/ fractions showed relatively higher zone inhibition in gram positive bacteria than gram negative bacteria at similar concentrations. This might be due to higher activity of the extract/ fractions on gram positive bacteria as most plant extract/ fractions were more active in gram positive bacteria [34, 35]. This difference may be explained by the difference in the structure of the cell wall in gram positive bacteria which consists of a single layer and the gram negative bacteria, which is a multi-layered structure and quite complex[36].

In the majority of test bacterial, the methanol extract and n-butanol fraction showed better activity. The higher activity of these extract and fraction might be associated with the number of bioactive metabolites and their synergetic activities. In the opposite, low activity of n-hexane and dichloromethane fractions might be due to the presence of lower quantity of metabolites detected. Therefore, the overall antibacterial effect of plant extracts might due to the presence of concentrated bioactive compounds or due to synergistic activity of two or more active metabolites.

Table1. Preliminary phytochemical screening of the crude methanol extract and solvent fractions of the leaves of *Dovyails abyssinica*

	Fractionation of solvents			
Secondary metabolites	Crude	n-hexane	Dichloromethane	n-butanol
Alkaloids	+	+	+	+
Flavonoids	+	-	-	+
Saponins	+	-	-	+
Tannins	+	+	+	+
Steroids	-	-	-	-
Phenolic compounds	+	-	+	+
Anthraquinones	+	-	-	+
Quinones	+	+	+	-
Terpenoids	+	+	-	+
Cardiac glycoside	-	-	-	-

Observed + = Present - = Absent

Table 2. Antibacterial activities of crude methanol extract of the leaves of *Dovyails abyssinica* against gram positive bacteria (*Enterococcus faecalis*).

		Concentration of extracts Zone of inhibition in diameter (mm)				
Test bacteria	Solvents& controls	45µg/well	90µg/well	900µg/well	(+) control	(-) control
E. faecalis	Crude methanol	7.3±0.9	10.3±0.5	12±0.8	22±0.0	NA

Values are expressed as mean ± SEM (n=3).NA= no activity (+) = positive control (Ciprofloxacin (5.0 µg/well) and (-) control = negative control (DMSO).

Table 3. Antibacterial activities of crude methanol extract and solvent fractions of the leaves of *Dovyails abyssinica* against gram positive bacteria (*Staphylococcus aureus*)

		Concentration of extracts				
		Zone of inhibition in diameter (mm)				
Test bacteria	Solvents & controls	45µg/well	90µg/well	900µg/well	(+) control	(-) control
S.aureus	Crude methanol	6.7±0.9	11.3±0.5	14.0±0.8	28±0.0	NA
	n-hexane	7.3±0.5	10.3±0.9	12.7±0.5	28±0.0	NA
	Dichloromethane	6.3±0.5	9.3±0.9	11.7±0.5	28±0.0	NA
	n-butanol	6.2±0.2	10.3±0.9	13.7±0.5	28±0.0	NA

Values are expressed as mean ± SEM (n=3). NA=no activity (+) = positive control (Ciprofloxacin (5.0 µg/well) and (-) control = negative control (DMSO).

Table 4. Antibacterial activities of crude methanol extract and solvent fractions of the leaves of *Dovyails abyssinica* against gram negative bacteria (*Escherichia coli*)

		Concentration of extracts				
		Zone of inhibition in diameter (mm)				
Test bacteria	Solvents	45µg/well	90µg/well	900µg/well	(+) control	(-) control
E.coil	Crude methanol	6.3±0.1	8.1±0.1	9.7±0.5	32±0.0	NA
	n-hexane	6.1±0.1	7.3±0.5	9.3±0.5	32±0.0	NA
	Dichloromethane	NA	7.3±0.5	10.3±0.5	32±0.0	NA
	n-butanol	6.0±0.2	9.3±0.5	11.0±0.8	32±0.0	NA

Values are expressed as mean ± SEM (n=3). NA= no activity (+) = positive control (Ciprofloxacin (5.0 µg/well) and (-) control = negative control (DMSO).

Conclusion

From the above results, it can be concluded that the traditional medicinal plant, the leaves *Dovyails abyssinica* has a certain degree of efficacy against the test bacterials. The results clearly indicated that using the leaves *Dovyails abyssinica* extract had the beneficial effect in controlling the bacterial infections.

References

(1) Moses, A.G. Maobe.; Erastus, Gatebe.; Leonard, Gitu.; Henry, Rotich. Preliminary Phytochemical Screening of Eight Selected Medicinal Herbs Used for the Treatment of Diabetes: Malaria and Pneumonia in Kisii Region, Southwest Kenya. *European Journal of Applied Sciences*.**2013**, *5*, 01-06.

(2) Degu, L.Keshebo.; Alemayehu, P. Washe.; Fikadu ,Alemu. Determination of Antimicrobial and Antioxidant Activities of Extracts from Selected Medicinal Plants.*ASRJETS*.**2016**,*16*(1), 212-222.

(3) Worku, Abebe. An Overview of Ethiopian Traditional Medicinal Plants Used for Cancer Treatment. *EJMP*.**2016**, *14*(4), 1-16.

(4) Kedarnath, N.K.; Surekha, R.S.; Mahantesh, S.P.; Patil,C.S. phytochemical screening and antimicrobial activity of *Aloe vera* L. *World Research Journal of Medicinal & Aromatic Plants*.**2012**, *1*(1), 11-13.

(5) Reta, Regassa. Diversity and conservation status of some economically valued indigenous medicinal plants in Hawassa College of Teacher Education Campus, Southern Ethiopia. *IJAR*. **2013**,*1*(3),308-328.

(6) E, Lulekal.; J,Rondevaldova.; E,Bernaskova.; J, Cepkova.; Z, Asfaw.; E,Kelbessa.; L,Kokoska.; P,VanDamme. Antimicrobial activity of traditional medicinal plants from Ankober District, North Shewa Zone, Amhara Region, Ethiopia. *Pharm Biol*.**2013**,1–7.

(7) Getachew, Alebie.; Befikadu, Urga .; Amha, Worku. Systematic review on traditional medicinal plants used for the treatment of malaria in Ethiopia: trends and perspectives. *Malar J*. **2017**,*16*,307.

(8) Abiyu,Enyew.; Zemede,Asfaw.; Ensermu,Kelbessa.; Raja,Nagappon. Status of medicocultural commercial plants at fiche town market, Ethiopia. *Int.J.Pharm&H.Care Res*.**2013**,*01*(04),227-236.

(9) Tomas, A.S.; Andrea, Salas.; Stewart, Reed.; Hamide, Gubbuk.; Michael, Winterstein. Physico-chemical Evaluation of *Dovyalis. Proc. Fla. State Hort. Soc.* **2014**,*127*,14–17.

(10) Van Vuuren, S.F.;Viljoen , A.M. The in vitro Antimicrobial Activity of Toothbrush Sticks Used in Ethiopia. *South African Journal of Botany*. **2006**, *72*(4): 646-648.

(11) Kiamba, J.K.; Schmidt, L.; Mbora, A.*Dovyalis abyssinica* (A. Rich) Warb. *Seed leaflet*. **2009**

(12) Chirchir,D.K.; Seriana,M.M.; Cheruiyot,Gilbert.; Adongo,J.O. Antimicrobial activity and phytochemical investigation of crude extracts of the fruits of *Solanum incanum* (Solanaceae) and *Dovyails abyssinica* (Flacourtiaceae). *Sjmb*. **2014**,*4*,193.

(13) Azene, B.Tesemma.; Ann, Birnie.; Bo, Tengnas.Useful Trees and Shrubs for Ethiopia; Identification, Propagation and Management for Agricultural and Pastoral Communities. Regional soil conservation unit,**1993**.

(14) Kimutai, Nicholas. *In-vitro* cytotoxicity of three selected medicinal plant extracts from Kenya.*WJPPS*.**2017**, *6*(6), 144-152.

(15) Aberra, Geyid.; Dawit, Abebe.; Asfaw, Debella.; Zewdneh, Makonnen.; Frehiwot, Aberra.; Frehiwot, Teka.; Tesfaye, Kebede.; Kelbessa, Urga.; Kidist, Yersaw.; Teklele, Biza.; Bisrat, H. Mariam.; Mulugeta,

Guta. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *Journal of Ethnopharmacology*. **2005**, *97*, 421–427.

(16) Kemboi, Doglas. Review of traditionally used medicinal plants by the Kipsigis community in Kenya.*BJPR*.**2016**, *12*(5), 1-6.

(17) Hesbon,O.Nyang'au.; John,Maingi.;Anthony,Kebira.The efficacy of some medicinal plants used locally within transmara west , Narok County, Kenya against selected enterobacteria and candida.*IOSR-JPBS*.**2017**,*12*(1),115-122.

(18) Gabriel, Kigen.; Fatuma, Some.; John, Kibosia.; Hillary, Rono.; Ezekiel, Kiprop.; Bernard, Wanjohi.; Prisca, Kigen.; Wilson, Kipkore.Ethnomedicinal Plants Traditionally Used by the Keiyo Community in Elgeyo Marakwet County, Kenya. *J Biodivers Biopros Dev*.**2014**,*1*(3).

(19) Kaleab, Asres.; Solomon, T.Esubalew.; Anteneh, Belete.; Ermias, Lulekal.; Tesfaye, Gabriel.; Ephrem, Engidawork. Review of Ethnobotanical and Ethnopharmacological Evidences of some Ethiopian Medicinal Plants traditionally used for the Treatment of Cancer. *Ethiop. J. Health Dev.* **2017**,*31* (3),161-187.

(20) Chepkwony, P.K.; Maria, Medina.; Mitchell, Medina. Compounds and compositions for treating infection. *Patent application publication*.**2012**

(21) Workineh, Shibeshi.; Belay, Tadesse.; Getachew, Terefe.; Nigatu, Kebede. In Vivo anti trypanosomal activity of dichloromethane and methanol crude leaf extracts of *Dovyalis abyssinica* (Salicaceae) against Trypanosomacongolense.*BMC Complementary and Alternative Medicine*. **2015**, *15*,278.

(22) Maxwell, O. Egua .; Emmanuel,U.Etuk.; Shaibu, O .Bello.; Sanusi, W. Hassan.Antidiabetic potential of liquid-liquid partition fractions of ethanolic seed extract of *Corchorus olitorious. JJP*. **2004**, *6*(1), 4-9.

(23) Jehan, Bakht.; Maria, K.P.; Mohammad, Shafi. Antimicrobial potential and phyto chemical analysis of different solvent extracted samples of *Viola pilosa*.*Pak. J. Bot*.**2017**, *49*,(4),1485-1489.

(24) Evans, W.C. Harcourt Brace and Company, Asia, Singapore. *Pharmacology*.**1997**, 226-22.

(25) Kumara, S. M.; Sudipta, K.M.; Lokesh, P.; M, Neeki. A.; Rashmi, W.; S, Bhaumik. H.;S ,Darshil. H.; R, Vijay.; SSN, Kashyap. Phytochemical screening and in vitro antimicrobial activity of *Bougainvillea spectabilis* flower extracts. *International Journal of Phytomedicine*. **2012**, *4* (3) 375-379.

(26) Neethu, Letha.; Kumar, Ganesan.; Suresh, K. P. N.; Henok, G. A.; Sharmila, B, G. Studies on phytochemical screening and *in vitro* Antioxidant activity of ethiopian indigenous Medicinal plants, *Artemisia abyssinica* sch.bip. Ex A.rich. *World Journal of Pharmaceutical Research*.**2016**,*5*(1),1048-1058.

(27) Foluke, Odutayo.; Cajethan, Ezeamagu.; Taofikat, Kabiawu.; Daniel, Aina.; Grace, M.A. Phytochemical Screening and Antimicrobial Activity of *Chromolaena odorata* Leaf Extract against Selected Microorganisms. *JAMPS*. **2017**,*13* (4), 1-9.

(28) P, Ganesh.; R, S. Kumar ,; P,Saranraj. Phytochemical analysis and antibacterial activity of Pepper (*Piper nigrum* L.) against some human pathogens. *Cent. Euro. J. Exp. Bio.* **2014**,*3* (2),36-41.

(29) Ali, Al Ghasham.; Mohammed, Al Muzaini.; Kamal, A.Qureshi.; Gamal,O.Elhassan.; Riyaz, A. Khan.;Syeda ,A. Farhana.; Sana, Hashmi.; Elsayed, El-Agamy.; Wail, E. Abdallah. Phytochemical Screening, Antioxidant and Antimicrobial Activities of Methanolic Extract of *Ziziphus mauritiana Lam.* Leaves Collected from Unaizah, Saudi Arabia. *IJPRPM*. **2017**, *6*(3), 33-46.

(30) Kabir,O.O.;Damilola,B.O.;Abdulfatai,A.A.;Akeem,A.J.Chemicalcomposition,antioxidant and antimicrobial potentials of *Icacina trichantha* olive leaf extracts.*Int.J.Trop.Med*.**2015**,*10*(4-6),21-29.

(31) Dey, S.K.; Banerjee, D.; Chattapadhyay,S.; Karmak, K.B .Antimicrobial activities of some medicinal plants of west Bengal. *Int J Pharm Sci.* **2010**, *1*, 1–9.

(32) Kuta, F.A.; Tsado, D.J.; Garba, S.A.; Saidu, A.N. Antibacterial activity of the leaf and stem bark crude extracts of Khaya senegalensis. *Med Aromat Plant Res J.* **2015**,*3*, 9–15.

(33) Emad, M. Abdallah,; Khalid, A. Al-Harbi. Phytochemical screening and antibacterial activity of crude aqueous and ethanol extracts of *Salvadora persica* L. stem (Miswak) from Saudi Arabia. *Jounrnal of Phytopharmacology*.**2015**,*4*(5),243-247.

(34) Doss,A.; Parivuguna,V.; Vijayasanthi,M.; Sruthi, Surendran. Antibacterial evaluation and phytochemical analysis of *Medicago sativa* L. against some microbial pathogens. *Indian Journal of Science and Technology*.**2011**,*4*(5).

(35) Elamathi, R.; Kavitha, R.; Kamalakannan, P.; Deepa, T.; Sridhar, S. preliminary phytochemical and antimicrobial studies on the leaf of *Ecbolium viride.WJMPBS*.**2012**,*2*(1),05-10.

(36) Jaradat, N.Amin.; Masound, Batool.; Abu-hadid, M.M. Screening antibacterial and antifungal activities and evaluation of the exhaustive extractions yields for *Verbascum sinuatum* L. *IJRAP*.**2015**,*6*(1).