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Assessment of the Antibacterial and Antioxidant Potentials of Mistletoe Grown on *Schinus molle* L., from Yegof National Forest Priority Area, South Wollo, Ethiopia

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ABSTRACT

Natural products derived from plant materials serve as potential sources of antimicrobial agents, and are very effective in preventing the destructive effects of oxidative stresses. The current study aimed to assess the antibacterial and antioxidant potential of solvent extracts of mistletoe (*Tapinanthus globiferus*) leaves. In this study, solvent system extraction and phytochemical screening were employed to detect the various bioactive phytochemical components. Disc diffusion antibacterial bioassay was conducted to determine the antibacterial activities of extracts. The radical scavenging activities of extracts were investigated using 2-diphenyl-1-picrylhydrazyl assay methods. During phytochemical screening, steroids, terpenoids, glycosides, alkaloids, flavonoids, tannins and anthraquinones were detected. Extracts having the concentration of 1 mg/mL revealed significant differences in their bioactivities ($p < 0.05$), with average inhibition zone diameters were ranging from 8.77 mm to 15.34 mm against the various target pathogens. Substantial average antibacterial activity (15.34 mm) was recorded by ethyl acetate extract against *Listeria monocytogenes*, a finding even greater than the positive control that showed an average inhibition zone diameter of (13.96 mm). But methanol extract revealed no antibacterial activity against most of the target pathogens tested, except on *Listeria monoctogenes* with average inhibition zone diameter of (11.54 mm). Most of the extracts showed no activity against *Klebsiella pneumonia*, except petroleum ether extract. On the other hand, solvent extracts showed promising antioxidant activities in the order of ethanol > ethyl acetate > petroleum ether. To evaluate the broader bioactivity spectrum of the extracts, wider ranges of pathogens including fungi must be considered. In addition, bioassay guided fractionation and identification of active molecules must be worked out further.

Keywords: Antioxidant and antibacterial activities, extraction, maceration, phytochemicals, *Tapinanthus globiferus*.

INTRODUCTION

The rapid spread of bacterial pathogens possessing multidrug-resistant properties (Andersson & Hughes 2010), and the emergence of new infectious agents threaten the health care system across the world. International organizations such as the US Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control and the World Health Organization are considering infections caused by multidrug-resistant bacteria and the spread of new pandemics as emergent global major public health concerns (Acharjee 2022; Valle et al., 2015; Ulloa-Urizar et al., 2015). These serious health challenges require urgent and integrated attention from academic researchers and all concerned bodies (Prestinaci et al., 2015; Kibret et al., 2018).

The emergence of new infectious agents such as SARS-CoV-2 (the causative agent of COVID 19), the alarming global distribution of multidrug-

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resistant pathogens, coupled with high cost and more side effects of synthetic antibiotics, have had led the interest of researchers and the general population towards ethno-medicinal plants for the potential discovery of useful antimicrobial compounds (Mlozi, 2022; Sen et al., 2021). Ethno-medicinal plants are those that humans have used as traditional medicine since ancient times. The herbal medications and health care preparations in widespread use, including those portrayed in antique texts such as Bible, have been traced as some of the best examples for the development of natural products with medicinal characteristics (Fernandez-Agullo, et al., 2013; Sanchez-Burgos, et al., 2013). Medicinal plants are indispensable for human wellbeing and provide a significant number of remedies required by health care systems. Thus, plant materials from various sources represent the vast natural pharmacies by virtue of their potentially enormous and untapped application for indigenous medicine (Bodeker et al., 2003).

Around the world, many plant materials are used as traditional medicines to cure various infectious diseases such as urinary tract infections, bronchitis, diarrhea, cutaneous abscesses and parasitic diseases, etc. Furthermore, plant-based traditional medicine plays a key role in the development and advancement of modern medicine by serving as a starting point for the development of novelties in drug discovery studies (Sasidharan et al., 2011; Weli, et al., 2022). Drugs developed from plant origin are not only successful in the management of infectious diseases but also are free from many of the side effects that are frequently associated with synthetic counterparts, and prevent various oxidative stresses (Kaneria et al., 2009; Kaneria & Chanda, 2013; Valle, et al., 2015).

In Ethiopia, traditional methods of dealing with illness have been practiced for centuries. The practice to a large extent focuses on the use of herbs, spiritual healings, bone-settings, and minor surgical procedures. Depending on the local customs, the practice varies in its form, procedure, and content (Arebu et al., 2017). It is estimated that more than 85% of traditional medical preparations in Ethiopia are of plant origins. Thus, in spite of an increase in the health service coverage of the nation, studies have reported that traditional medicine continues to play an important role in healthcare throughout the country (Arebu et al., 2017). The Ethiopian Ministry of Health in Ethiopia has given very serious attention to medicinal plants used by the local people. However, the loss of valuable medicinal plants due to lack of documentation, underreporting, agricultural expansion, urbanization, and deforestation has been widely reported by various researchers. Documentation of data regarding medicinal plants is of great value to facilitate the discovery of new sources of drugs and promote sustainable use of the nation's natural resources. It is also vital for health service planning to incorporate the best herbal medicines as alternatives in a country's health care delivery system (Harborne &Williams, 1992; Arebu et al., 2017).

In this regard, *Tapinanthus globiferus* (*T. globiferus*) commonly known as mistletoe and locally named as *Ye Quando Berberie Teketla* in Amharic (Ethiopian national language) is widely used in different forms as a traditional treatment for various ailments by the local people around Kombolcha in South Wollo administration zone, Ethiopia. *T. globiferus* is a perennial evergreen shrub that grows as a hemi-parasite on woody plant species such as *Schinus molle* (*S. molle*), a pepper tree (*Quando Berberie* in Amheric), as host plant. Studies have showed that when grown on other various host plants this hemi-parasitic plant contains large amounts of bioactive compounds (Son et al., 2010; Beuth et al., 2008; Pietrzak et al., 2017). Its bioactivities and antioxidant properties when grown on *S. molle* have not been reported scientifically in the study area yet. Therefore, the aim of the current study was to evaluate the antimicrobial and antioxidant potentials of the different solvent extracts of this epiphytic plant material against selected human pathogens such as *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13889), *Listeria monocytogenes* (clinical isolate), and *Staphylococcus aureus* (ATCC 25923). The work may also serve as a stepping stone for further study in this area.

MATERIALS AND METHODS

Sampling site and sample collection:

Plant material was collected from Yegof National Forest Priority Area, which is located in South Wollo administration zone on a steep mountain ridge overlooking Kombolcha town, 395 km north of Addis Ababa. It is composed of a natural highland forest mostly consisting of dry evergreens, mixed conifers, broadleaved trees, and plantations of fast-growing exotic trees. Interestingly, the forest is dominated by *Juniperus procera*. It is under intense stress from settlement, land-use conversion to farming and grazing, deforestation, and neglect in terms of forest conservation (Sultan & Berhanu, 2013; Ahmed et al., 2022). Yegof is situated between 11° 01' to 11° 03' N latitude and 39° 40′ to 39° 44' E longitude with an elevation of between 2000 m and 3014 m above sea level (Fig. 1). The annual average temperature of the study area varied from 8.5° C to 39.9° C. The reported average maximum rainfall was 1036 mm. The area has a mean annual minimum and maximum temperature of 12.7°C

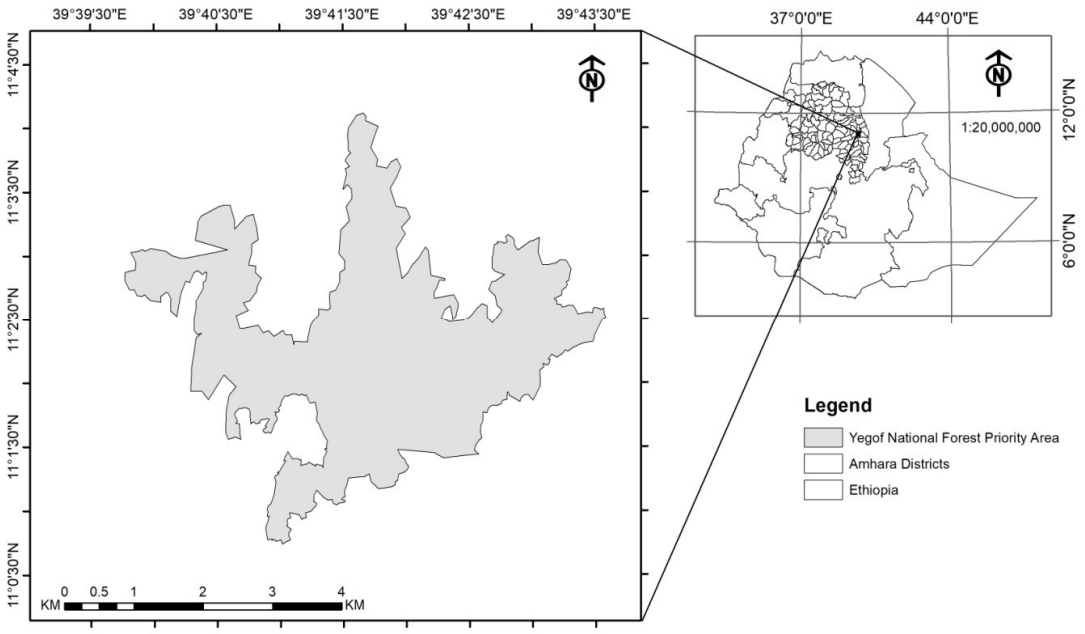

Fig. 1: Yegof National Forest Priority Area, the location of sample collection

and 27.1°C, respectively. The forest supports a wide variety of wild animals including Erckel's francolin (*Pternistis erckelii*), Menilik's bushbuck (*T. scriptus* meneliki), wild pig (*Sus scrofa*) etc. (Goudar, et al., 2017; Mohammed et al., 2017; Ahmed et al., 2022).

The experimental plant material, *T. globiferus* leaves were collected from the study area aseptically. The botanical specimens of the host plant (*S. molle*) and *T. globiferus* (the epiphyte), were collected, pressed, dried, identified and deposited in Wollo University herbarium, Dessie, Ethiopia. The botanical identification was further confirmed by botanists in the Department of Biology, Wollo University. Before processing, the samples were washed under running tap water to eliminate dust/dirt and other foreign particles and to cleanse the plant parts thoroughly, and then dried at room temperature. The selected plant materials were cut into tiny pieces, dried under the shade and packed into sealable plastic bags and labeled at the chemistry department laboratory, Wollo University.

Extraction of plant material and extract preparation:

The plant material, once air-dried, was coarsely ground into a powder using an electric grinder. This resulted in 150 g of powdered material. This powder was then divided into three equal parts and each part was placed in a 500 mL round-bottom flask. Each flask was filled with 300 mL of a different solvent (methanol, ethyl acetate, and petroleum ether, respectively). The flasks were then placed in a rotary shaker and left to macerate for 72 hours at a temperature of 25ºC. The extracts were filtered first by Whatman filter paper number 1 with a pore size of 11µm to obtain the crude extract solutions. The filtrate was centrifuged at 2000 rpm for about 10 min. The crude extract solution from each solvent was concentrated to a minimum volume by using a rotary evaporator at 45°C under reduced pressure. The dried extracts were stored at 4°C until chemical analysis and bioactivity test were performed as described in Anokwuru, (2011).

Phytochemical Screening and Thin layer Chromatography analysis:

Screening of important phytochemical classes in the extracts such as steroids, terpenoids, glycosides, saponins, alkaloids, flavonoids, tannins, phlobatann, and anthraquinone were investigated following standard protocols described (Harborne, 1973; Boham & Kocipai, 1994; Obadoni & Ochuko, 2001; Ngameni et al., 2013; Poumale et al., 2013; Wansi et al., 2013).

Thin layer chromatography analysis was conducted using different developing solvent systems so as to evaluate the chemical profile of the extracts. Thin layer chromatography was conducted using Silica gel 60 F254 Multi-format pre-scored to 20 x 20 cm aluminum coated plates (stationary phase). Different mobile phases consisting of a mixture of hexane, chloroform and ethyl acetate with different ratios were used. Thin layer chromatography plate starting lines were drawn in pencil at 1.5 cm from the bottom edge; giving an elution distance of maximal 4 cm. Spots were placed on the plate with a small capillary tube, placed in thin layer chromatography jar and run through a series of solvent systems. UV detection at (254 nm and 360

nm) was carried out by UV light source. Chromatograms were further developed by vanillin/H_2SO_4 reagent. After the development of the chromatograms, the thin layer chromatography plates were completely sprayed with a solution of the dyeing reagent and heated with a heating plate at 110° C until the colored spots appeared. The spots were visually detectable after color development at room temperature. Finally, the chemical profile of each extract was determined by calculating their retention factor (Rf value) relative to the solvent front.

Target pathogens and antibacterial bioassay:

The current study targeted multidrug-resistant bacterial pathogens such as *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13889), *Listeria monocytogenes* (clinical isolate), and *Staphylococcus aureus* (ATCC 25923). The target pathogens were obtained from the Ethiopian public health institute and Amhara regional laboratory (Dessie branch). The isolates were cultured with nutrient agar medium and stored with slants at 4° C until used. During the bioassay, crude extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain a test concentration of 1 mg/mL (by dissolving 100 mg of the plant material in to 100 ml of the solvent). Disc diffusion bioassay method described in Clinical Laboratory Standard Institute (2012) was employed for antibacterial susceptibility testing. The turbidity of the inoculum suspensions was adjusted to a 0.5 McFarland standard determined by the optical density reading at 625 nm. The inoculum density for each target pathogen was adjusted to a final concentration of 2 x 10^8 CFU/mL. 100µL of each suspension was inoculated on the entire surface of Mueller-Hinton agar plates with sterile cotton swabs inside a laminar air flow cabinet. Filter paper discs (6 mm in diameter, impregnated with 100 µL of the 1 mg/mL crude extract) were completely dried and placed on the surface of agar plates seeded with the target pathogens. Standard chloramphenicol discs (30 µg/mL) were used as positive control and discs impregnated with the solvent dimethyl sulfoxide (DMSO) was used as negative control (Clinical Laboratory Standard Institute, 2012).

Antioxidant activity (2-diphenyl-1-picrylhydrazyl radical scavenging assay) of extracts:

The antioxidant activity of *T. globiferus* extract obtained using ethyl acetate, petroleum ether and ethanol was evaluated using 2-diphenyl-1-picrylhydrazyl (DPPH) assay as described in Yen & Chen (1995). Different concentrations (300, 250, 200, 150, 100 and 50 µg/mL) of plant extracts and 0.3 mM of 2-diphenyl-1-picrylhydrazyl were made using methanol as a solvent. Two mL of each series concentration of plant extracts were mixed with 1 mL of 0.2 mM of 2-diphenyl-1-picrylhydrazyl. Then the mixture was shaken vigorously and kept in a dark room for 30 min. The absorbance was recorded in the range of 400-600 nm by using DPPH as a negative control and methanol as a blank. The free radical scavenging activity was estimated using the following expression:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_t)/A_0) \times 100$$

Where, A_0 is the absorbance of the control and A_t is the absorbance of the sample.

Method of data analysis:

All experiments were conducted in triplicates, the data were analyzed using one-way ANOVA and the mean separation was achieved by the Duncan's multiple range tests, using SPSS (version 22). Numerical differences were considered as statistically significant at $p \leq 0.05$.

RESULTS

Phytochemicals and thin layer chromatography profiles of extracts:

Depending on the geographical location of a plant, its growth habit and the type of host plant, both the quality and quantity of an active component (most often secondary metabolites) of various epiphytic plants might differ considerably. The qualitative analysis indicated that the major bioactive phytochemical classes were detected in ethyl acetate and methanol extracts, including steroids, terpenoids, glycosides, alkaloids, flavonoids, tannins and anthraquinones (Table 1). Similarly, thin layer chromatography analysis of crude extracts using silica gel 60 F254 plates revealed several bands (5-8) represented by different retention factors (Rf-values) (Fig. 2). The different bands observed showed the presence of a mixture of important bioactive substances in ethyl acetate and methanol extracts.

Antibacterial activities of crude extracts:

The study evaluated the antibacterial activities of various solvent extracts (ethyl acetate, methanol and petroleum ether) from *T. globiferus* leaves. These leaf extracts demonstrated a range of average antibacterial inhibition zone diameters from 8.77 mm to 15.34 mm against the various target pathogens, with the most noteworthy results recorded for the ethyl acetate extract. In this study, extracts exhibited statistically significant differences in their bioactivities against the target pathogens ($p < 0.05$). Although there were lower antibacterial activities found against some gram-negative bacterial pathogens tested, ethyl acetate extract showed prominent bioactivity with an average inhibition zone diameter of 15.34 mm against *Listeria monocytogene* (clinical isolate) a finding even better than the positive control

chloramphenicol (13.39 mm) against the same pathogen. The finding indicates that the mean inhibition zone diameter observed by 1 mg/mL of ethyl acetate extract were 10.77, 9.77, 15.34 and 12.52 mm against *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13889), *Listeria monocytogenes* (clinical isolate), and *Staphylococcus aureus* (ATCC 25923), respectively (Table 2). On the other hand, methanolic extract showed no antibacterial activity against most of the target pathogens tested, except on *Listeria monoctogenes* (clinical isolate) with average inhibition zone diameter of (11.54 mm). Similarly, most of the extracts showed no activity against Klebsiella pneumonia (ATCC 13889), while the petroleum ether extract that revealed an average inhibition zone diameter of 9.77 mm.

Antioxidant activity (DPPH radical scavenging assay):

Antioxidant capacities of extracts (petroleum ether, ethyl acetate and ethanol) were investigated based on the DPPH assay method using ascorbic acid as standard. The analysis revealed that the 2-

Table 1: Major phytochemical classes detected from the crude extracts during qualitative analysis

Analysis approach	Target phytochemical class	Observation	Remarks
Salkowski's test	*Steroids and Terpenoids*	Appearance of red color in the lower layer and formation of reddish-brown color at the interface after addition of conc.H_2SO_4	indicates the presence of steroids and terpenoids, respectively
Keller Killiani's test	Glycosides	Formation of two layers, the lower reddish-brown layer and upper acetic acid layer which turns bluish green	indicates a positive test for glycosides
Hager's test	Alkaloids	Yellow precipitate formation	indicates the presence of alkaloids
Lead acetate solution test	Flavonoids	Formation of yellow precipitate	indicates the presence flavonoids
Gelatin Test	Tannins	White precipitate formation	indicates the presence of tannins
Ferric chloride test	Tannins	An intense green, purple, blue or black color	indicates the presence of tannins
Anthraquinones test	Anthraquinones	Appearance of red color	indicates the presence of anthraquinones

Table 2: Bioactivity profiles of extracts using standard bioassay method

Extracts	**Average inhibition zone diameters of extracts (in mm) against the target pathogens:**			
	Escherichia coli	*Klebsiella pneumonia*	*Listeria monocytogenes*	*Staphylococcus aureus*
Ethyl Acetate	10.77	-	15.34	12.52
Methanol	-	-	11.46	-
Petroleum ether	8.77	9.77	-	-
Positive control	17.79	18.30	13.96	28.17
Negative control	-	-	-	-

- No activity

Table 3: Percent scavenging of crude extracts and standards

Concentration (μg/mL)	Pet ether (%)	EtOAc (%)	EtOH (%)	Ascorbic Acid (Control) (%)
50	51.41	66.31	62.45	94.57
100	81.98	72.32	87.37	94.85
150	82.2	81.59	88.56	95.13
200	91.44	88.70	92.20	95.29
250	92.78	89.75	92.99	95.57
300	93.85	91.44	93.12	95.81

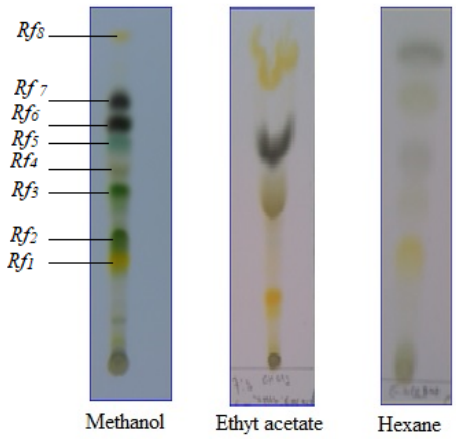

Fig. 2: Bands obtained during thin layer chromatography analysis of crude extracts of mistletoe from *S. molle*

diphenyl-1-picrylhydrazyl radical inhibition activities were enhanced with increasing the quantity of *T. globiferus* extracts; this indicates the presence of antioxidants in a solution mixture that interact with the free radicals. In ethyl acetate, petroleum ether and ethanol extracts, concentrations varied as (50, 100, 150, 200, 250 and 300 µg/mL) so as to assess the dose-dependent scavenging activities. The finding indicated that the antioxidant activities of the crude extract increase with increasing concentration regardless of the solvent type used. The maximum wave length recorded in each experiment was 517 nm as revealed by the Ultraviolet–Visible Spectroscopy data (Fig. 3).

In terms of maximum antioxidant activity recorded, the most efficient sample was ethanol extract. Its potential was significantly higher ($p < 0.001$) than that of ethyl acetate and petroleum ether extracts (Fig. 3). Increasing the concentration of each extract resulted in increasing the ability of scavenging of the free radicals (Table 3). At the initial concentration (50 µg/mL), ethyl acetate extract showed better inhibition activity (66.31%) than did petroleum ether (51.14%) and ethanol extracts (62.54%). However, as the concentration of each crude extract was raised to 100 µg/mL, the inhibition potential of ethanol extract dramatically increased to 87.37%, while scavenging activity of ethyl acetate extract increased to 72.32% and that of petroleum ether to 81.98% (Fig. 4).

DISCUSSION

The emergence of new contagious agents such as SARS-CoV-2 (the causative agent of COVID 19) and the rapid rise and spread of multidrug-resistant bacterial pathogens present a serious global medical crisis, one that highlights the need to search for alternative antimicrobial agents. Therefore, efforts have to focus on the

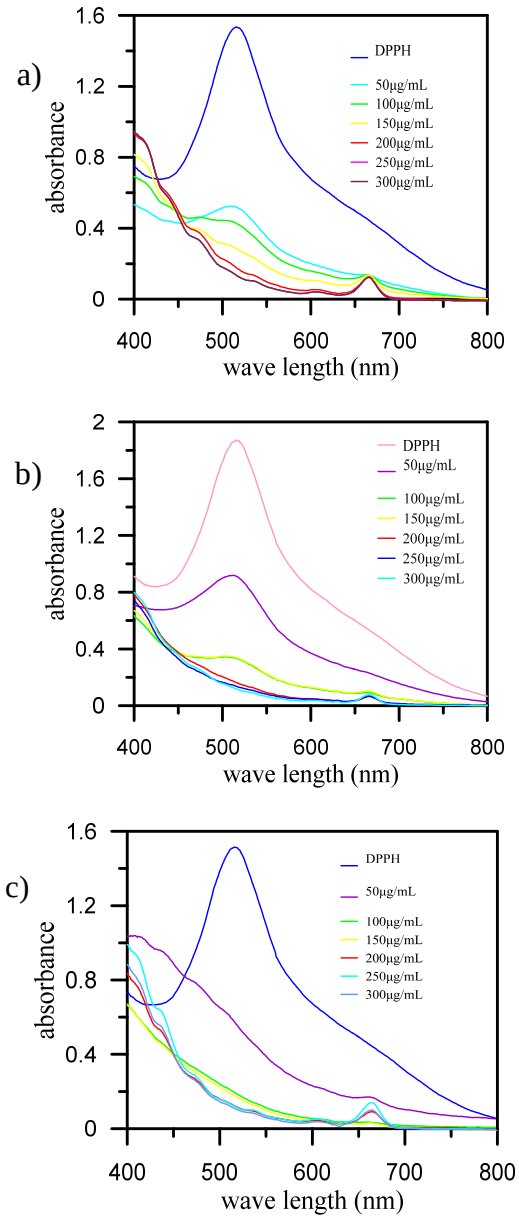

Fig. 3: Free radical scavenging activity of mistletoe extracts at different solvents a) ethyl acetate b) petroleum ether c) ethanol

development of potential therapeutic agents from all available sources. In the current study, the various phytochemical classes analyzed and detected were secondary metabolites biosynthesized in *T. globiferus* in which promising antimicrobial efficacies were found. This might be attributed to the availability of various bioactive compounds in the solvent extracts of mistletoe that were rich in a variety of phytochemical classes including flavonoids, tannins, anthraquinones, alkaloids, steroids and terpenoids. On the other hand, the findings revealed that these secondary metabolites could be isolated by organic solvents, which are suitable for the extraction of bioactive compounds from plant materials. Thus, the current

findings strengthen the significance of the analyzed plant material (a medicinal plant used by the local people) as a promising source of antimicrobial compounds against important gram-positive bacterial pathogens such as *Staphylococcus aureus* (ATCC 25923) and *Listeria monoctogenes* (clinical isolate). Likewise, previous studies on various medicinal plants including Panda et al. (2020), Ulloa-Urizar et al. (2015) and Al-Daihan et al. (2013) reported similar results.

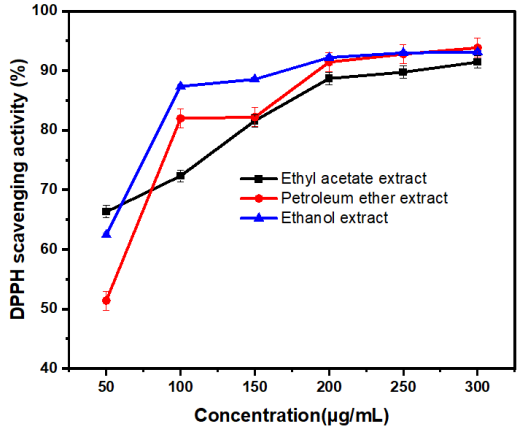

Fig. 4: DPPH scavenging activity of crude extracts

Fig. 5: A) The host plant *S. molle* L. (Pepper tree), B) The parasitic plant *T. globiferus* (Mistletoe) from *S. molle*

The detection of various bands ranging from (5-8) during the thin layer chromatography analysis, with different Rf values indicated that the antimicrobial activities of the crude extract could be due to the presence of various bioactive molecules that act singly or synergistically with different modes of actions. In relation to the current findings, reports from previous studies by thin layer chromatography fingerprinting showed that medicinal plants are rich in vital phytochemical classes such as alkaloids, flavonoids, phenols, glycosides, steroids, saponins and terpenoids (Panda et al., 2020).

Antimicrobial bioassay data revealed that ethyl acetate and methanolic extract exhibited substantial antibacterial activity against *Listeria monoctogenes* (clinical isolate). However, none of the crude extracts showed bioactivity against Klebsiella pneumonia (ATCC 13889) except

Fig. 6: A) powder form of mistletoe of *Schinusmolle*, B) the crude extract

petroleum ether extract, which exhibited an average inhibition zone diameter of 9.77 mm. This might be due to the solubility difference of the bioactive substance in the different solvent systems having different polarities. The finding demonstrated that Gram-positive strains were more sensitive to the antimicrobial activities of the extracts than Gram-negative strains. This might be due to the fact that the outer membrane found in the Gram-negative cell wall is composed of structural lipopolysaccharides that render the cell wall impermeable to the available antimicrobial substances while Gram-positive counterparts lack this structure to prevent the entrance of the various bioactive substances in the extract. Hence, such structural differences influence their reaction to the antibacterial agents. Several similar studies on different medicinal plants (Yasunaka et al., 2005; Al-Daihan et al., 2013; Chanda et al., 2013; Valle et al., 2015; Jdey et al., 2017; Razafintsalama et al., 2017; Sagbo et al., 2017; Acharjee 2022), also reported various levels of antibacterial activities consistent with the current findings. The result showed that the extract from *T. globiferus* exhibited promising activities against gram-positive bacterial pathogens. In this regard, Prado et al. (2019) also reported the antimicrobial potentials of the host plant (*S. molle*). This implies that the epiphyte and the host plant have some sort of relationship in their secondary metabolite biosynthesis pathways.

The antioxidant activities of three solvent extracts (ethyl acetate, petroleum ether and ethanol) of mistletoe were conducted using DPPH assay because of its simplicity and access. DPPH is a stable free radical compound and has an absorbance in its oxidized form around 515-520 nm. The antioxidant activities of *T. globiferus* extracts were assessed in terms of absorbance intensity decline as compared to the blank. The color changes observed on the addition of extracts indicated the scavenging activities by addition of hydrogen, since DPPH is able to accept an electron or hydrogen radical to form a stable diamagnetic molecule. The current investigation clearly demonstrated the strong antioxidant activities of extracts, (ethyl acetate, petroleum ether, ethanol), a finding consistent with that of Jdey et al. (2017). The DPPH radical inhibition activities were enhanced with the increasing concentration of all solvent extracts of *T. globiferus*, (ethyl acetate, petroleum ether, and ethanol). This indicates that the antioxidants found in a solution mixture interact with free radicals. Similarly, Abebe et al. (2022) who assessed the antioxidant activity of the aqueous leaf extracts of Combretum microphyllum and the effect of Co(II)-leaf extract reported that scavenging activity increased with increasing concentration of the extracts.

In ethyl acetate, petroleum ether and ethanol extracts, concentrations varied (50, 100, 150, 200, 250 and 300 μg/mL) to assess its dose-dependent scavenging activities. The radical scavenging activities recorded for those extracts were higher at higher concentration. The better quenching rate of diphenylpicrylhydrazyl radicals showed greater antioxidant ability of the obtained solvent extracts in the order of ethanol > ethyl acetate > petroleum ether. The result exhibited that ethanol extract displayed better antioxidant activity, which implies that polar solvents are known to extract the antioxidants substances. Similarly, a report by Sagbo et al. (2017) on the antioxidant properties of two medicinal plants reported in similar fashion.

The finding revealed that extraction methodology is important in the antioxidant assay. This implies that the yield of the extract depend on the polarity of solvent used during preparation (Sun et al., 2005). Moreover, solubility of the natural products and the choice of solvent could also determine the antioxidant activities (De Monte et al., 2014). The result interestingly indicated that extracts of *T. globiferus* are important sources of natural antioxidant metabolites. The antioxidant ability of such extracts might be related with reducing power, superoxide scavenging capacity and donating a hydrogen atom or preventing peroxide formation (Kumaran & Karunakaran, 2006).

In conclusion, the study revealed that *T. globiferus* extracts, which have been used by local people as a traditional medicine demonstrated promising antimicrobial activities against various drug resistance pathogens, but bioactivities varied depending on the solvent type used for extraction. The antioxidant activities of extracts were found to be dose dependent. The polar solvent (ethanol) extract displayed better antioxidant activity. To look for the broad-spectrum bioactivities of extracts, various target pathogens including fungi should be included in the antimicrobial bioassay work. Literature is inadequate on phytochemical analysis of *T. globiferus* in the study area. Therefore, attention must be given to documenting and preserving this medicinal plant material for further drug development studies. Further, bioactivity guided fractionation and identification of the active components must be worked out in the future.

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Data availability statement:

Data are available from corresponding author on reasonable request.

Declarations:

Ethics approval and consent to participate:

The researchers have discussed about the relevance of the study and have got the consent of the respective Kebele administration office before plant material was collected from Yegof National Forest Priority Area.

Consent for publication:

The authors have provided their consent for publication.

Competing interests:

The authors declare that they have no competing interests.

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