OPEN ACCESS

Scholars International Journal of Chemistry and Material Sciences

Abbreviated Key Title: Sch Int J Chem Mater Sci
ISSN 2616-8669 (Print) |ISSN 2617-6556 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://saudijournals.com

Original Research Article

Phytochemical Screening and Antimicrobial Activity of Stem Bark Extracts of *Schinus molle linens*

Temesgen Bekele[1*], Biruk Bezabeh Yimam[2]

[1]Department of Chemistry, College of Natural Science, Wollo University, Dessie, Ethiopia
[2]Department of Chemistry, College of Natural Science, Mizan- Tepi University, Tepi, Ethiopia

DOI: 10.36348/sijcms.2023.v06i05.002 | **Received:** 07.07.2022 | **Accepted:** 12.08.2022 | **Published:** 26.05.2023

***Corresponding author:** Temesgen Bekele
Department of Chemistry, College of Natural Science, Wollo University, Dessie, Ethiopia

Abstract

The majority of the world population prepares medicines from plants to cure diseases. Schinus molle linens is a multipurpose plant that has been used for various remedies as constituents of traditional medicine. The aim of this study was to investigate the chemical constituents and antimicrobial activity of methanol extract and solvent fraction extracts of the stem bark of Schinus molle. Powder of Schinus molle was extracted with methanol by using the maceration method and the fractions were obtained by successive fractionation of the methanol extract with n-hexane, chloroform, ethyl acetate, and n-butanol. Preliminary phytochemical analysis of the crude methanol extract revealed the presence of flavonoids, terpenes, anthraquinones, alkaloids, tannins, phenols, and saponins. In addition to this, the antimicrobial activity of crude and solvent fraction extract was tested against S. aureus, E. faecalis, E. coli, and C. albicans at a concentration of 90.0, 180.0, and 900.0 µg/ mL. The crude methanol extract and chloroform fraction exhibited greater activity against gram-positive, gram-negative bacteria and fungi. The n-hexane and n-butanol fractions showed the least activity against most test bacteria and fungi. The study showed that it is possible to inhibit the growth of bacteria and fungi at different concentrations.

Keywords: Phytochemical screening**,** antimicrobial activity, *Schinus molle*, Agar well diffusion.

1. INTRODUCTION

Any plant possessing chemicals that can be used for therapeutic purposes and are precursors for the development of effective medicines from one or more of its components is considered a medicinal plant [1]. The medicinal herb is extremely beneficial to human health. It has been used as an anti-bacterial agent against bacterial diseases since ancient times [2]. Medicinal plants are known to produce some compounds that are naturally harmful to organism, and they are inexpensive and renewable sources of pharmacologically active molecules [3].

Schinus molle belongs to the Anacardiaceae family [4-23] and is commonly called pepper fruits [4], Peruvian Pepper Tree [5, 11, 17], California's pepper tree [6, 7], Brazilian pepper tree [14] American pepper tree [17], false pepper [17, 18] pink pepper [6, 18]. It is native to the American continent [7], whereas it is distributed worldwide. The plant is locally known as Kundo Berbere (Amharic) [8].

Schinus molle plant can reach up to 10 meters high. It has thin, long leaves. At the correct season, its fruit resembles small berries (3-6 mm across) and turns red or bluish-pink as they mature. Its small white flowers are arranged in drooping branched clusters [7, 14].

Schinus molle. is used to treat analgesic, central depressant [6], plague [7], asthma, dysentery, hemorrhage, laryngitis , sore throat, spasm, trachagia, vermifuge, ulcers, gout, diarrhea [9], antispasmodic, skin disease [7,9],antibacterial, topical antiseptic, stimulant, antidepressant [6, 15], astringent [7, 15], digestive [6, 15, 17], wound healing [6, 7, 9, 15], respiratory, urinary infections [6,9,15], purgative diuretic, toothache, rheumatism, menstrual disorders [6, 9, 15, 17], colds, coughs [9, 16] fractures, to stop bleeding, insecticidal activity [15] tuberculosis, bronchitis, fever [16], and intestinal parasites [22].

There is no literature review that can show the phytochemical screening in the stem bark of *Schinus*

molle composition of methanol crude extract and solvent fraction integrally to inhibit selected pathogenic microbial.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The fresh stem bark of *Schinus molle* was collected from Dessie town, Amhara regional state, Ethiopia.

2.2. Experimental site

Extraction, phytochemical screening, and fractionation were conducted at Wollo University, Department of chemistry. The bacterial activity test was done at Amhara Regional Health Research Laboratory Center, Dessie Town, in the Hotie district.

2.3. Preparation of plant material

The fresh stem bark parts of *Schinus molle* were first cleaned by removing the dead external parts by rubbing with clean hands and then washing with tap water. It was cut using an axe into small pieces to facilitate drying and allowed to dry in the shaded area. The dried stem bark was powdered with the electronic grinder and the coarse and fine powders were sorted by using a sieve. The powder sample was weighed and kept in airtight containers until the extraction process began [24, 25].

2.4. Extraction

The powdered plant material (300.0 g) was macerated in 1500.0mL of methanol in a conical flask covered with aluminum foil at room temperature for about 72 h. The macerated material was shaken at regular intervals of time. The supernatant was filtered through Whitman No.1 filter paper with gravity filtration. The filtrate was concentrated using a rotary evaporator at 40^{o}C and stored in a refrigerator at 4^{o}C until use [24, 25].

2.5. Solvent Fractionation

The methanol crude extract (15.0 g) was mixed in100.0 mL of distilled water and stirred, transferred into a separatory funnel. It was fractionated sequentially in100.0 mL of n-hexane, chloroform, ethyl acetate, and n-butanol. Each fractionation was repeated three times. Each organic extract was dried with anhydrous sodium sulfate and concentrated under reduced pressure by using a rotary evaporator. The dried extracts were kept in the refrigerator at 4 ℃ until needed [25, 27].

2.6. Phytochemical screening

Preliminary qualitative phytochemical analyses were conducted on the crude extract and solvent fractions.

Test for flavonoids

The extract (0.5 mL) was transferred into the test tube and added four drops of 10% $FeC1_3$ solution. The formation of a green precipitate indicates the presence of flavonoids [28].

Test for Terpenoids

2.0 mL of chloroform was added to the extract (1.0 mL). 3.0 mL of concentrated sulfuric acid was then gently added, forming a layer. Terpenoids were present because a reddish-brown tint developed [25,28].

Test for anthraquinones

Shaken with 10.0 mL of benzene, the extract (1.0 mL) was then filtered. The filtrate was then mixed with 0.5 mL of a 10 percent ammonia solution. Anthraquinones were present because a violet color developed [25, 28].

Test for alkaloids

The extract (1.0 mL) was treated with 2.0 drops of Wagner's reagent (1.27g iodine and 2.0g potassium were dissolved in 100.0 mL distilled water) to form a yellow precipitate which indicates the presence of an alkaloid [28].

Test for Tannins

10.0 mL of distilled water was added to the extract (1.0 mL), mixed, and then filtered. The filtrate was added to this solution along with 3.0 drops of a 10% ferric chloride reagent. Anthraquinones were known to generate a violet color when present [25, 28].

Test for Phenols

The extract (1.0 mL) was treated with 5% $FeC1_3$ solution. The formation of the Dark green color was the presence of phenols [28].

Test for Quinones

Concentrated hydrochloric acid was used to treat the extract (1.0 mL). The formation of green color was the presence of quinones [28].

Test for Saponin

The extract (1.0 mL) was dissolved in 6.0 mL of distilled water in a test tube. The mixture was shaken vigorously. The formation of Persistent foam under was the presence of Saponin [28].

Test for Oxalate

The extract (1.0 mL), 2.0 mL of distilled water was added and treated with 1.0 mL of concentrated hydrochloric acid. The formation of a greenish-black was the presence of oxalate [29].

2.7 In vitro antimicrobial studies

2.7.1 Antimicrobial test organisms and standard antimicrobial disc

The standard American Type Cell Culture (ATCC) bacterial species of *Escherichia coli* (gram-negative), *Staphylococcus aureus*, and *Enterococcus faecalis* (gram-positive), and the fungal species was *Candida albicans* were obtained from the Amhara Public Health Institute–Dessie Branch Clinical Bacteriology and Mycology Laboratory, Dessie City,

Ethiopia. The standard antibacterial and antifungal discs used for the study were ciprofloxacin and nystatin.

2.7.2. Determination of zone of inhibition

The antimicrobial activities of plant extract were tested using the agar well diffusion method [32]. About 70.0 mL of freshly prepared sterile Mueller Hinton Agar (bacteria) and Potato dextrose agar (fungus) media were poured into a 150.0 mm diameter agar plate and allowed to cool at room temperature. Within 15 minutes of reaching the 0.5 McFarland criterion for turbidity, a sterile cotton swab was placed into the modified microbial suspension, gently swirled, and firmly pressed on the inside wall of the tube above the fluid level to remove additional inoculum from the swab. The swab was streaked to the entire surface of the MHA/PDA plate three times by rotating approximately 60^{o} each time to ensure even distribution of the inoculum. Petri-plates were left for 5 minutes at room temperature [33] and then an equal distance hole with a diameter of 6 mm was punched aseptically using a sterile cork borer tip. The extracts (0.5 g) were dissolved in1.0mL DMSO to give 0.5 g/mL stock solution. Different concentrations of the extracts (90 – 900 µg/mL) were prepared by diluting the stock solutions in DMSO [25, 26]. Ciprofloxacin and DMSO were used as the negative and positive control, respectively. Fixed volume (90. 0 µL) of the extracts and the controls were introduced into the labeled wells. After placement of the plant extracts and controls into labeled wells, the plates were left undisturbed at room temperature for 2 h. The plates were then incubated at 37 $^{\circ}C$ for 24 h [25, 26]. On the other hand, for fungal species, nystatin (100 units/ disc) was used as a positive control. Similar to bacterial test organisms, DMSO was used as a negative control. Then, Petri-plate was incubated at 30 ºC for 48 hours [34].

By utilizing the ruler and the naked eye, the entire zone of inhibition was measured in millimeters. For each bacterial species, each test was carried out in triplicate. For the solvent fractions and methanol extract as well as for typical antibacterial and antifungal discs, the mean zone of inhibition and standard error of the mean (Mean SEM) are computed.

2.8. Data Analysis

The percentage yield of the crude extract solvent fractional was calculated with the formula:

$$\text{Percentage yield of crude} = \frac{Crude\ of\ extract}{dry\ weight\ of\ the\ samples} \text{ x } 100$$

$$\text{Percentage yield of fractions} = \frac{weight\ of\ the\ fractionated\ extract}{Weight\ of\ the\ methanol\ extract} x100$$

Phytochemical screening test results were reported as present (+) or absent (-). For the antibacterial efficacy test, the mean zone of inhibition is expressed as mean and standard error of the mean (Mean ± SEM) for each triplicate determination.

3. RESULT AND DISCUSSION

The yield of the crude methanol and fractionation of stem bark extract of *Schinus molle* is presented in Table 1. The percentage yield of crude methanol extract was 14.6% methanol extraction. The chloroform had the highest percentage yields compared to another solvent fractionation but n-hexane had the least percentage yields as shown from shown in Table 1.

3.1 Phytochemical screening results of methanol extract and solvent fraction

Preliminary phytochemical analysis of the crude methanol extract revealed the presence of flavonoids, terpenes, anthraquinones, alkaloids, tannins, phenols, and saponins in stem bark extract of *Schinus molle* and the absence of oxalates and quinine was investigated in the sample extract by different test methods (Table 2). The result is in agreement with the findings of Gonzalo *et al*., [6], who reported terpenes were present. Additionally, the result is in line with the findings of Lamboro *et al*., [8], who reported flavonoids, alkaloids, and phenols were present and quinones were absent by using chloroform. Differences in results might be related to the geographical distribution of the plant and extraction methods used [30]. Alkaloids were detected in both methanol extract and each solvent fraction. Oxalates and quinine were absent in each extract. While, terpenes and phenols were present in chloroform, ethyl acetate, and n-butanol fraction. Flavonoids, tannins, and saponins had only chloroform and n-butanol respectively. Amongst all fractions, the chloroform fraction appeared to be relatively rich in secondary metabolites as shown from shown in Table 2.

3.2. Antimicrobial susceptibility assay

Antimicrobial activities of both crude methanol extract and each solvent fraction (n-hexane, chloroform, ethyl acetate) were evaluated using the agar well diffusion method at concentrations of 90.0, 180.0, and 900.0 µg/mL as shown in Tables 3, 4 and 5.

Among the test bacteria, the maximum average zone of inhibitions at 900.0 µg/mL concentration in gram-positive bacterial species was determined to be 17.3±0.5 mm for *E. faecalis* and 18.0±0.5 mm for *S. aureus*. On the other hand, the maximum average inhibitions, at a similar concentration in gram-negative bacteria species was 10.3±0.9 mm for *E.coil*. On the contrary, no zone of inhibition was observed at all concentrations of n-hexane and 90.0 µg/mL ethyl acetate fraction against *E.coil*.

The zone of inhibition of the crude methanol extract at 900.0 µg/mL concentration extract was greater than that of other fraction extracts against *S.*

aureus and *E. faecalis*. Similarly, at 90.0 and 180.0 µg/mL of equal concentration, the zone of inhibition of the chloroform fraction was greater than that of extracting other fractions and crude methanol against *S. aureus.* At 90.0 µg/mL concentration zone of inhibition of the crude methanol extract and chloroform were equal, against *E. faecalis* (Table 3). Furthermore, the zone of inhibition of the chloroform fraction at 180.0 µg/mL concentration was greater than that of crude methanol extract at equal concentration, against *E. coil* (Table 3).

The crude methanol extract shows greater antibacterial activity in stem bark extract of *Schinus molle* against gram-positive bacterial test (*E. faecalis* and *S. aureus*). The result is in agreement with the findings of Ricardo *et al*., [10], who reported *E. faecalis* and *S. aureus* were inhibited by using methanol. Furthermore, the antibacterial activity by using crude methanol extract was inhibited against *E. coil* and *S. aureus.* The result is in agreement with Salem *et al*., [9], who reported the bark of extract of *Schinus molle* was detected in *S. aureus*, and *E. coil* by using methanol.

For antifungal activity, the crude methanol and solvent extract were determined for extracts that have shown different zone of inhibition as shown in Table 5. The crude methanol and chloroform extract of stem bark extract of *Schinus molle* at 900.0 µg/mL concentration were determined to be 11.4±0.8 mm and 10.3±0.9 mm respectively. The result is in agreement with the findings of Ricardo *et al*., [10], who reported *Candida albicans* was inhibited by using methanol. The *Schinus molle* plant has shown antifungal activity based on the different concentrations that are explained in different literature [7-11, 14, 18, 21, 23]. On the contrary, no zone of inhibition was observed at all concentrations of n-hexane and 90.0 µg/mL ethyl acetate and n-butanol fraction against *Candida albicans*. In addition, at 180.0 µg/mL concentrations of ethyl acetate fraction was not show a zone of inhibition.

The present study showed that solvent fraction and crude methanol of stem barks of *Schinus molle* extract could be able to relatively higher zone inhibition in pathogenic bacteria and fungi at similar concentrations. Therefore, the overall antimicrobial effect of plant extracts might due to the presence of concentrated bioactive compounds or due to the synergistic activity of two or more active metabolites.

Table 1: The yield of crude methanol extract and fractionation steam bark of *Schinus molle*

No	Types of extract	% Yield
1	Crude methanol extract	14.6
2	n-hexane fraction	11.3
3	Chloroform fraction	14.0
4	Ethyl acetate fraction	12.7
5	n-butanol fraction	11.8

Table 2: Preliminary phytochemical screening of the crude methanol extract and solvent fractions of the stem bark extract of *Schinus molle*

No	Secondary metabolites	Crude Extracts	Fractionation of solvents			
			n-hexane	Chloroform	ethyl acetate	n-butanol
1.	Flavonoids	+	-	+	-	-
2.	Terpenes	+	-	+	+	+
3.	Anthraquinones	+	+	-	-	-
4.	Alkaloids	+	+	+	+	+
5.	Tannins	+	-	+	-	-
6.	Phenols	+	-	+	+	+
7.	Quinones	-	-	-	-	-
8.	Saponins	+	-	-	-	+
9.	Oxalates	-	-	-	-	-

Observed + = Present - = Absent

Table 3: Antibacterial activities of crude methanol extract and solvent fractions of stem bark extract of *Schinus molle* against gram-positive bacteria

		Concentration of extracts Zone of inhibition in diameter (mm)				
Test bacteria	Solvent and fraction	90.0 µg/mL	180.0 µg/mL	900.0 µg/mL	(-) control	(+) control
S. aureus	crude methanol	10.2±0.1	14.1±0.5	18.0±0.5	-	23.0
	n-hexane fraction	8.6±0.1	9.6±0.9	12.5±0.5	-	23.0
	Chloroform fraction	11.2±0.9	14.8±0.1	15.7±0.5	-	23.0
	Ethyl acetate fraction	9.1±0.1	12.3±0.2	14.2±0.9	-	23.0

	n-butanol fraction	10.0±0.4	12.2±0.6	15.2±0.1	-	23.0
E. faecalis	crude methanol	10.3±0.1	14.1±0.9	17.3±0.5	-	21.4
	n-hexane fraction	7.3±0.1	9.3±0.5	11.2±0.5	-	21.4
	Chloroform fraction	10.3±0.1	13.3±0.5	15.2±0.5	-	21.4
	Ethyl acetate fraction	10.2±0.7	11.5±0.3	13±08	-	21.4
	n-butanol fraction	8.0±0.3	10.3±0.5	11.3±0.1	-	21.4

Values are expressed as mean ± SEM (n=3) - = 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 stem bark extract of *Schinus molle* against gram-negative bacteria

		Concentration of extracts Zone of inhibition in diameter (mm)				
Test bacteria	**Solvent and fraction**	**90.0 µg/mL**	**180.0 µg/mL**	**900.0 µg/mL**	**(-) control**	**(+) control**
E.coli	crude methanol	6.7±0.1	7.5±0.0	9.0±0.8	-	24.0±0.0
	n-hexane fraction	-	-	-	-	24.0±0.0
	Chloroform fraction	6.1±0.1	8.1±0.1	10.3±0.9	-	24.0±0.0
	Ethyl acetate fraction	-	6.7±0.9	8.6±0.3	-	24.0±0.0
	n-butanol fraction	6.2±0.3	7.1±0.5	9.6±0.7	-	24.0±0.0

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

Table 5: Antifungal activities of methanol extract and solvent fractions of the stem bark extract of *Schinus molle* against *Candida albicans*

		Concentration of extracts Zone of inhibition in diameter (mm)				
Test bacteria	**Solvent and fraction**	**90.0 µg/mL**	**180.0 µg/mL**	**900.0 µg/mL**	**(-) control**	**(+) control**
Candida albicans	crude methanol	7.0±0.1	9.5±0.0	11.4±0.8	-	19.0±0.0
	n-hexane fraction	-	-	-	-	19.0±0.0
	Chloroform fraction	6.2±0.1	8.1±0.1	10.3±0.9	-	19.0±0.0
	Ethyl acetate fraction	-	-	7.3.±0.4	-	19.0±0.0
	n-butanol fraction	-	7.1±0.5	9.6±0.7	-	19.0±0.0

Values are expressed as mean ± SEM (n=3) - = no activity (+) = positive control (nystatin) and (-) control = negative control (DMSO).

4. CONCLUSION

The above findings indicate that *Schinus molle* stem bark, a traditional medicinal plant, has some effectiveness against the test bacteria and fungi. On the studied bacterial and fungal species, the crude methanol extract and the other solvent fractions (n-hexane, chloroform, ethyl acetate, and n-butanol) displayed varying degrees of bacterial and fungal activity. Depending on the type of the organism, certain of these fractions were more efficient than the others. Among the solvent, crude methanol extract and chloroform were the most active in gram-positive, negative bacteria and fungi. These activities may be attributed to the presence of bioactive secondary metabolites including flavonoids, terpenes, anthraquinones, alkaloids, tannins, phenols, and saponins that act either individually or collectively to bring about the overall antimicrobial effect. From the findings of this study, it is possible to conclude that the *Schinus molle* stem bark can prevent bacterial and fungi infection in both crude and other solvent fractions.

Conflicts of interest: The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS

The author would like to thank the Wollo University, Ethiopia for their support.

REFERENCES

1. Okigbo, R. N., Anuagasi, C. L., & Amadi, J. E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of medicinal plants Research*, *3*(2), 86-95.
2. Dangeubun, J. L., & Metungun, J. (2017). Hematology of Vibrio alginolyticus-infected humpback grouper Cromileptes altivelis, under treatment of Alstonia acuminata shoot extract. *Aquaculture, Aquarium, Conservation & Legislation*, *10*(2), 274-284.
3. Bandaranayake, W. M. (2006). Quality control, screening, toxicity, and regulation of herbal drugs. *Modern phytomedicine: turning medicinal plants into drugs*, 25-57.
4. Cherroud, S., El Yaacoubi, A., Abba, E. H., & Ainane, T. Essential Oil of Wild Pepper Fruits (Schinus Molle) from Morocco: Chemical Composition and Biological Activities.

5. Kienel, U., Bowen, S. W., Byrne, R., Park, J., Böhnel, H., Dulski, P., ... & Negendank, J. F. (2009). First lacustrine varve chronologies from Mexico: impact of droughts, ENSO and human activity since AD 1840 as recorded in maar sediments from Valle de Santiago. *Journal of Paleolimnology*, *42*(4), 587-609.
6. Malca-García, G. R., Hennig, L., Ganoza-Yupanqui, M. L., Piña-Iturbe, A., & Bussmann, R. W. (2017). Constituents from the bark resin of Schinus molle. *Revista Brasileira de Farmacognosia*, *27*, 67-69.
7. Silva-Júnior, E. F., Aquino, P. G. V., Santos-Júnior, P. F. S., Nascimento, I. J. S., Gomes, E. A., Silva, A. L. L., ... & Araújo-Júnior, J. X. (2015). Phytochemical compounds and pharmacological properties from Schinus molle Linnaeus and Schinus terebinthifolius Raddi (Anacardiaceae). *Journal of Chemical and Pharmaceutical Research*, *7*(12), 389-393.
8. Lamboro, T., Mengistu, M., & Hordofa, T. G. (2020). Phytochemical screening, characterization of essential oil and antimicrobial activity of Schinus molle (Anacardiaceae) collected from Eastern Hararghe, Ethiopia.
9. Salem, M. Z., Zayed, M. Z., Ali, H. M., El-Kareem, A., & Mamoun, S. M. (2016). Chemical composition, antioxidant and antibacterial activities of extracts from Schinus molle wood branch growing in Egypt. *Journal of Wood Science*, *62*(6), 548-561.
10. Salazar-Aranda, R., Pérez-Lopez, L. A., Lopez-Arroyo, J., Alanís-Garza, B. A., & Waksman de Torres, N. (2011). Antimicrobial and antioxidant activities of plants from northeast of Mexico. *Evidence-Based Complementary and Alternative Medicine*, *2011*.
11. Abdul-Hafeez, E. Y., Ibrahim, O. H. M., Mahmoud, A. F., & Abo-Elyousr, K. A. M. (2016). Effect of Schinus molle and Schinus terebinthifolius extracts on sweet pea damping-off. *Assiut J Agric Sci*, *47*, 63-74.
12. Gehrke, I. T., Neto, A. T., Pedroso, M., Mostardeiro, C. P., Da Cruz, I. B., Silva, U. F., ... & Morel, A. F. (2013). Antimicrobial activity of Schinus lentiscifolius (Anacardiaceae). *Journal of ethnopharmacology*, *148*(2), 486-491.
13. Bouzada, M. L., Fabri, R. L., Nogueira, M., Konno, T. U., Duarte, G. G., & Scio, E. (2009). Antibacterial, cytotoxic and phytochemical screening of some traditional medicinal plants in Brazil. *Pharmaceutical Biology*, *47*(1), 44-52.
14. Onder, D., Artun, S., Nedim, T., & E, E. H. K. (2010). Chemical composition, repellent and antimicrobial activity of Schinus molle L. *Journal of Medicinal Plants Research*, *4*(21), 2211-2216.
15. Ferrero, A. A., Werdin González, J. O., Sánchez Chopa, C., & Alzogaray, R. A. (2006). Biological activity of Schinus molle on Triatoma infestans. *Fitoterapia*, *77*(5), 381-383.
16. Guerra-Boone, L., Álvarez-Román, R., Salazar-Aranda, R., Torres-Cirio, A., Rivas-Galindo, V. M., de Torres, N. W., ... & Pérez-López, L. A. (2013). Chemical compositions and antimicrobial and antioxidant activities of the essential oils from Magnolia grandiflora, Chrysactinia mexicana, and Schinus molle found in northeast Mexico. *Natural Product Communications*, *8*(1), 1934578X1300800133.
17. Tahtamouni, R. W. (2018). Investigating the Antibacterial Potential of Ethanolic and Methanolic Extracts of the Schinus molle L Tree. *Jordan Journal of Biological Sciences*, *11*(5).
18. Turchetti, G., Garzoli, S., Laghezza Masci, V., Sabia, C., Iseppi, R., Giacomello, P., ... & Ovidi, E. (2020). Antimicrobial testing of Schinus molle (L.) leaf extracts and fractions followed by GC-MS investigation of biological active fractions. *Molecules*, *25*(8), 1977.
19. Belhamel, K., Abderrahim, A., & Ludwig, R. (2008). Chemical composition and antibacterial activity of the essential oil of Schinus molle L. grown in Algeria. *International Journal of Essential Oil Therapeutics*, *2*(4), 175-177.
20. Machado, D. G., Kaster, M. P., Binfaré, R. W., Dias, M., Santos, A. R., Pizzolatti, M. G., ... & Rodrigues, A. L. S. (2007). Antidepressant-like effect of the extract from leaves of Schinus molle L. in mice: evidence for the involvement of the monoaminergic system. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *31*(2), 421-428.
21. Machaba, T. C., & Mahlo, S. M. (2017). Antifungal activity of medicinal plants used traditionally for the treatment of fungal infections and related ailments in South Africa. *International Journal of Pharmacological and Pharmaceutical Sciences*, *11*(6), 395-400.
22. Zenebe, S., Feyera, T., & Assefa, S. (2017). In vitro anthelmintic activity of crude extracts of aerial parts of Cissus quadrangularis L. and leaves of Schinus molle L. against Haemonchus contortus. *BioMed research international*, *2017*.
23. Alonso, M. M., Salazar, J. C. L., Robles, S. O., Guerrero, I. C., García, F. L., & Marrero, J. G. (2020). In vitro antimicrobial activity of mexican plants on bovine mastitis bacteria: preliminary studies. *Bioscience Journal*, 183-190.
24. Mulatu, G. (2020). Antibacterial activities of Calpurnia aurea against selected animal pathogenic bacterial strains. *Advances in Pharmacological and Pharmaceutical Sciences*, *2020*.
25. Legesse, B. A., Tamir, A., & Bezabeh, B. (2019). Phytochemical screening and antibacterial activity of leaf extracts of Dovyalis abyssinica. *J Emerg Technol Innov Res*, *6*(6), 453-465.
26. Kassaw, S., Tamir, A., & Yimam, B. B. (2022). Phytochemical Investigation and Determination of Antibacterial Activities of the Fruit and Leaf Crude

Extract of Ficus palmata. *Sch Int J Chem Mater Sci*, *5*(4), 61-66.
27. Bakht, J., Panni, M. K., & Shafi, M. (2017). Antimicrobial potential and phyto chemical analysis of different solvent extracted samples of viola pilosa. *Pak. J. Bot*, *49*(4), 1485-1489.
28. Shaikh, J. R., & Patil, M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608.
29. Ugochukwu, S. C., Uche, A., & Ifeanyi, O. (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. *Asian journal of plant science and research*, *3*(3), 10-13.
30. Abdallah, E. M., & Al-Harbi, K. A. (2015). Phytochemical screening and antibacterial activity of crude aqueous and ethanol extracts of Salvadora persica L. stem (Miswak) from Saudi Arabia. *J. Phytopharm*, *4*(5), 243-247.
31. Amin, J. N., Batool, M., & Mahmoud, M. A. (2015). Screening antibacterial and antifungal activities and evaluation of the exhaustive extractions yields for Verbascum sinuatum L. *Int J Res Ayurved Pharma*, *6*(1), 105-110.
32. Sen, A., & Batra, A. (2012). Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. *Int J Curr Pharm Res*, *4*(2), 67-73.
33. Dey, S. K., Banerjee, D., Chattapadhyay, S., & Karmakar, K. B. (2010). Antimicrobial activities of some medicinal plants of West Bengal. *International Journal of Pharma and Bio Sciences*, *1*(3), 1-10.
34. Tofighi, Z., Molazem, M., Doostdar, B., Taban, P., Shahverdi, A. R., Samadi, N., & Yassa, N. (2015). Antimicrobial activities of three medicinal plants and investigation of flavonoids of Tripleurospermum disciforme. *Iranian Journal of Pharmaceutical Research: IJPR*, *14*(1), 225.