International Journal of Tropical Medicine

MEDWELL PUBLICATIONS

Physicochemical Properties and Biological Activities of Common Mullein (*Verbascum thapsus* L.) Root, Leaf and Fruit Oils

Zekeria Yusuf, Giorgis Hailu, Mulugeta Desta and Sewnet Mengistu
School of Biological Sciences and Biotechnology, Haramaya University, Dire Dawa, Ethiopia

Key words: DPPH, factorial design, Minimum Inhibitory Concentration (MIC), oil extract, zone of inhibition

Corresponding Author:
Zekeria Yusuf
School of Biological Sciences and Biotechnology, Haramaya University, Dire Dawa, Ethiopia

Page No.: 47-52
Volume: 16, Issue 4, 2021
ISSN: 1816-3319
International Journal of Tropical Medicine

Abstract: *Verbascum thapsus* L. is one of the most common medicinal plant. The purpose of this study was to examine physicochemical properties, antioxidant and antimicrobial activities of oil extracted from common mullein leaves, roots and fruits. The oil extraction was done in Soxhelt apparatus using hexane as a solvent. The result of physicochemical properties of *V. thapsus* fruit, leaf and root oils presented significantly the highest oil yield (17.75%) was recorded for the fruit oil extract. Acid value (acid number) (2.53) and free fatty acid (FFA) (1.27) value were significantly highest for root oil. Specific gravity (0.92) and peroxide values (2.20) were highest for leaf oil. Significantly the highest antioxidant activities with respect to DPPH (37.35±0.64) was recorded for root oil. The strongest antibacterial activity with maximum zone of inhibition (14.50 mm), minimum inhibitory concentration (MIC, 0.19 µL/mL) and corresponding minimum bactericidal concentration (MBC, 0.25 µL/mL) was recorded for root oil extract against *Staphylococcus aureus* while the strongest antifungal activity with maximum zone of inhibition (16.50 mm), MIC (0.09 µL/mL) and MFC (0.18 µL/mL) were recorded for fruit oil against *Aspergillus niger*. Hence, suggesting possible exploitation of these plants for their antimicrobial active principles for the development of novel herbal-based antimicrobials.

INTRODUCTION

Common mullein belongs to Scrophulariaceae family, also known as Wooly Mullein (*Verbascum thapsus* L.) is represented by 360 global species and has been used as a medicinal herb since time immemorial[1]. The leaves and flowers of this plant are reported to have expectorant and demulcent features which are used to treat various respiratory problems[2, 3]. The plant is also reported to be mildly diuretic and to have a soothing and anti-inflammatory action on the urinary tract and to act as a mild sedative. *V. thapsus* extract has also been used as a domestic remedy for ailments such as pneumonia, fever, congestion, allergies, migraine, catarrhs and colic[3]. Scrophulariaceae members are a source wide variety of chemical constituents e.g. saponins, monoterpene

glycosides, iridoids, phenylethanoid glycosides, neolignan glycosides, flavonoids, steroids, spermine alkaloids, phenolic acids and fatty acids[4].

Verbascum thapsus chemical constituents include: 3% mucilage that transforms to galactose, arabinose and aromatic acids after hydrolyzes; 4% flovonoeids including rutine and hesperidin as common (cause diuretic effects), triterpenesaponins, verbascosaponin(have expectorant activity), iridoid glycoside, tannen: acobin, katapol and related compounds have anti-inflammatory activity[5, 3]. Several *Verbascum* species have been indicated as having antioxidant, narcotic, antiseptic, emollient, astringent, expectorant, sedative and diuretic properties; moreover, they are used as a treatment for inflammations, tumors and migraine[6].

The ever-increasing resistance of human pathogens to the available antimicrobial agents is a serious threat, resulting in an urgent need for novel antibiotic resources such as plants[7]. Some species of the genus *Verbascum* have been used by mankind since ancient times to treat internal and external infections. The genus *Verbascum* has been tested for antimicrobial and antifungal activity, however, the results have revealed that the extracts of the different species of the genus *Verbascum* do not exhibit similar antimicrobial effects against different kinds of bacteria and fungi[2]. On account of such justification the present study was aimed to examine physicochemical properties, antioxidant and antimicrobial activities of oil extracted from common mullein leaves, roots and fruits.

MATERIALS AND METHODS

The experiment was conducted in Animal Nutrition Laboratory, Haramaya University. The common mullein plant sample was collected from Melkabello District, East Hararghe, Ethiopia. The plant sample was manually washed with distilled water and residual moisture was evaporated at room temperature. Thereafter, air dried and the root, leaves and fruit samples were chopped and ground in a grinder for 2 min, the process was stopped for 15 sec to avoid heating of sample. The moisture content of the samples were adjusted to 5% prior to oil extraction. The oil extraction was done in Soxhelt apparatus using hexane as a solvent. Then, physicochemical properties of the oil extracts were done based on determination of oil content, specific gravity, acid value, percent free fatty acid and peroxide values as per the standard procedure described by Association of Analytical Chemists[8]. The antioxidant activity was investigated based on determination of ascorbic acid content, DPPH and hydrogen peroxide free radical scavenging activities as per the procedure used by Association of Analytical Chemists[9].

Antimicrobial activity test: The antimicrobial activity experiment was arranged as 3×1×4 (3 oil extracts, 1 solvent system, i.e., hexane and 4 test microbes including two bacteria: *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive); two fungi: *Aspergillus versicolor* and *A. niger*) completely randomized factorial design in three replications. The test pathogens were obtained from Plant Pathology Laboratory, Haramaya University. The fungal and bacterial pathogens were sub cultured and maintained on Potato Dextrose Agar (PDA) and Nutrient Agar, respectively. Then, the fungal and bacterial cultures were incubated for 72 h at 27ºC and for 18-24 h at 37ºC, respectively.

Media preparation and standardization of inoculum: Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Muller Hinton agar (MHA) was used for sub-culturing of bacterial test organism, fungal test organism and determination of antimicrobial activities, respectively. These media were prepared and sterilized using an autoclave according to the manufacturers' instructions. Two to three bacterial colonies on the plate were picked up with a sterile inoculating loop and transferred into a test tube containing sterile normal saline and vortexes thoroughly. The spores of the test fungi were harvested by washing the surface of the fungal colony using 5mL of sterile saline solution. This procedure repeated until the turbidity of each bacterial and fungal spore suspension matched the turbidity of 0.5 McFarland Standards as described by the Clinical Laboratory Standards Institute[10]. The resulting suspension will be used as inoculums for the test pathogen in the antimicrobial susceptibility test using disc diffusion method[11].

Disc diffusion method: The discs of 6 mm diameter was prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then impregnated each of them was impregnated with 0.01 mL of the prepared test extract ethyl acetate solution. The extract impregnated discs were placed onto MHA plates evenly inoculated with test pathogens. Ketokonazole (1 µL/disc) disc was applied as positive control and distilled water served as negative control for incubation of fungi while ampicillin (1 µL/disc) was served as positive control and deionized water was served as negative control for bacterial pathogens. Then, the MHA plates were sealed with parafilm and incubated at 37°C for 24 hrs and 27°C for 72 h for bacterial and fungal pathogens, respectively. The diameters of the zone of inhibition around each disc were measured to the nearest millimeter along two axes (i.e., 90° to each other) using a transparent ruler and the means of the two readings were be recorded.

Determination of Minimum Inhibitory Concentration (MIC): The oil extracts of common mullein oil extracts that showed significant antimicrobial activity in the antimicrobial activity tests were selected for determination of MIC based on the method used by Morshed *et al*.[12] with slight modifications. The MIC of the oil extracts was determined by broth dilution method. Two milliliter of nutrient broth and potato dextrose broth for bacteria and fungi, respectively were added into all test tubes and 0.1 mL of the prepared concentration of each oil extract were mixed with the nutrient broth and potato dextrose. Thereafter, standardized inoculums of 0.1 mL of the respective test pathogens were dispensed into the test tubes containing the suspensions of the broth and the oil extract. Then, all test tubes were properly corked and incubated at 37°C for 24 h for bacteria and 27°C for 72 h for fungi. After that, they were observed for absence or presence of visible growth. The lowest concentration at which no visible growth of organisms were regarded as the MIC. The experiment was carried out for each test organism in triplicates.

Determination of Minimum Bactericidal (MBC) and Fungicidal Concentrations (MFC): For the determination of the MBC and MFC, fresh nutrient agar and potato dextrose agar plates were inoculated with one loop full of culture taken from each of the broth cultures that showed no growth in the MIC tubes. That is MBC/MFC values were determined by subculturing from respective MIC values. Since, antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC[10]. MBC/MFC is the amount of the extract that kills microbial growth. While MBC assay plates were incubated for 48 h, MFC assay plates were incubated for 3 days. After the incubation periods, the lowest concentration of the extract that did not allow any bacterial or fungal growth on solid medium was regarded as MBC and MFC for the extract[10]. This observation was matched with the MIC test tube that did not show evidence of growth after 48 h of incubation for bacteria or spore germination after 3 days of incubation for fungi.

The experimental data were analyzed using SAS version 9.2.[13] to investigate statistical significance between the different oil quality parameters. Differences between means were considered statistically significant at $p<0.05$ based on LSD (Least Significance difference) t-test.

RESULTS AND DISCUSSION

Physicochemical properties of common mullein (*Verbascum thapsus* L.) fruit, leaf and root oil extracts
The physicochemical properties of oil extracts from *Verbascum thapsus* fruit, leaf and root was evaluated using oil quality parameters as in Table 1. Significance differences were observed among the oil extracts except for peroxide values. Significantly the highest oil yield (17.75%) was recorded for the fruit oil extract. Acid value (acid number) (2.53±0.40) and free fatty acid (FFA) (1.27±0.20) value were significantly highest for root oil. Specific gravity (0.92±0.01) and peroxide values (2.20±0.28) were highest for leaf oil. According to FAO (2010), seeds that contain oil yield >17% are considered as oil seeds. The avocado pear seed is therefore not recommended for the purpose of edible oil generation and biodiesel production due to the very low oil yield. However, variation in oil yield may be due to the differences in species of plant, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method as well as solvent used.

Acid value is used to measure the extent to which glyceride in the oil has been decomposed by lipase and other actions such as light and heat. The lower the acid value of oil, the fewer free fatty acids it contains which makes it less susceptible to rancidity. Peroxide Value (PV) measures the degree of lipid oxidation in fats and oils but not their stability. PV measures a transient (temporary) product of oxidation. A low value may represent early or advanced oxidation which can be distinguished with time[14].

Antioxidant activities of *Verbascum thapsus* fruit, leaf and root oil extracts: The antioxidant activities of the oils extracted from *V. thapsus* fruit, leaf and root were evaluated based on ascorbic acid content, DPPH and hydrogen peroxide free radical scavenging activities as in Table 2. Significantly the highest antioxidant activities with respect to DPPH (37.35±0.64) was recorded for root oil followed by fruit oil (26.55±0.21) and the least for DPPH value (11.15±1.20) for leaf oil. By contrast, significantly the highest hydrogen peroxide free radical scavenging activities (HPSA, 25.45±0.78) was obtained for leaf oil. There was no significance differences obtained for ascorbic acid. The highest DPPH value (37.35) for root oil indicates the highest antioxidant activities and the presence of highest essential omega-3 fatty acids in *V. thapsus* root oil. The antioxidant activities of root oil was found to be significantly the highest indicating that root oil might possess better biological activities, oil quality and pharmacological applications in *V. thapsus*. This finding was in agreement with local Ethiopian folkloric medicine of *V. thapsus* root for treatment of abdomenal pain.

Antimicrobial activities of *Verbascum thapsus* fruit, leaf and root oil extracts: The antimicrobial activities based on diameter of zone of inhibition for *Verbascum thapsus* fruit, leaf and root oil extracts demonstrated

Table 1: Physicochemical properties of *Verbascum thapsus* fruit, leaf and root oil extracts

Oil extract	Oil content	Spgr	ACV	FFA	PV
Fruit	17.75±0.35a	0.61±0.04b	0.98±0.20b	0.49±0.10b	2.10±0.14a
Leaf	12.75±0.23b	0.92±0.01a	1.54±0.40b	0.78±0.09b	2.20±0.28a
Root	11.50±0.71b	0.53±0.03b	2.53±0.40a	1.27±0.20a	1.90±0.14a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row. Spgr: specific gravity; ACV: acid value; FFA: free fatty acids; PV: peroxide value

Table 2: Antioxidant activities of *Verbascum thapsus* fruit, leaf and root oil extracts

Oil extract	DPPH	HPSA	AA
Fruit	26.55±0.21b	13.65±0.64c	26.88±3.79a
Leaf	11.15±1.20c	25.45±0.78a	27.18±3.38a
Root	37.35±0.64a	17.70±0.85b	27.39±3.45a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row. DPPH: 2, 2- diphenyl-1-picrylhydrazyl; HPSA: hydrogen peroxide scavenging activity; AA: ascorbic acid

Table 3: Antimicrobial Activities oil extracts from *V. thapsus* fruit leaf and seed as mean diameter of zone of inhibition against test pathogenic microbes

		Concentration of the extract (v/v)			
Test pathogens	Oil extract	1 µL/mL	2 µL/mL	3 µL/mL	Ampicillin (1 µL/mL)
E. coli	Fruit	0.00±0.00dD	6.47±0.41dC	9.00±0.90cB	18.5±0.50aA
	Leaf	0.00±0.00dD	9.50±0.50cC	12.33±0.29bB	18.5±0.50aA
	Root	7.83±0.76cD	10.00±0.52cC	13.00±0.50aB	18.83±0.29aA
S. aureus	Fruit	8.80±0.72bcC	12.00±0.35bB	13.00±0.50aB	18.83±0.29aA
	Leaf	9.67±0.76abC	12.50±0.32bB	13.50±0.50aB	18.33±0.28aA
	Root	10.00±0.55aC	13.50±0.50aB	14.50±0.40aB	18.33±0.12aA
Ketokonazole (1 µL/mL)					
A. versicolor	Fruit	9.83±0.29bD	11.50±0.50cC	15.50±0.40aB	18.00±0.45aA
	Leaf	0.00±0.00eD	6.17±0.76eC	12.50±0.50bB	17.67±0.29aA
	Root	11.50±0.32aD	13.27±0.25bC	14.50±0.50aB	17.17±0.76aA
A. niger	Fruit	9.60±0.53bC	14.50±0.40aB	16.50±0.50aB	17.83±0.29aA
	Leaf	5.50±0.36dD	10.50±0.60dC	13.50±0.50bB	17.33±0.26aA
	Root	6.50±0.40cD	11.00±0.57cdC	14.77±0.25aB	17.50±0.50aA

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. Small letters: significance within column; capital letters: significance across row. *E. coli*: *Escherichia coli; S.aureus*: *Staphylococcus aureus*

considerable antimicrobial activities against test pathogensas in Table 3. The mean zone of inhibition at highest concentration (3 µL/mL) against bacterial test pathogens ranged from 9.00±0.90-14.50±0.40 mm while 12.50±0.50-16.50±0.50 mm against fungal test pathogens. The strongest antibacterial activity with maximum zone of inhibition (14.50 mm) at highest concentration (3 µL/mL) of the oil was recorded for root oil extract against *S. aureus* while the weakest antibacterial activity (9.00 mm) was observed for fruit oil against *E. coli* indicating that *S. aureus* was more susceptible. Hence root oil has exhibited most antibacterial potential than fruit and leaf oils in common mullein (*Verbascum thapsus* L.).

On the other hand, the strongest antifungal activity with maximum zone of inhibition (16.50 mm) was recorded for fruit oil against *A. niger* as the weakest antifungal activity with minimum zone of inhibition (12.50 mm) was recorded for leaf oil against *A. versicolor* suggesting fruit oil extract might be more effective antifungal potential than root and leaf oil extracts in *V. thapsus.* Similar study was conducted by Dulger *et al*.[15] who demonstrated the antimicrobial activities extracts from three *Verbascum* spp found that no significant activity found against Gram (-) bacteria. The distinctive feature of gram-negative bacteria is the presence of a double membrane surrounding each bacterial cell. Although all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than are gram-positive bacteria[16].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), Minimum Fungicidal Concentration (MFC) for common mullein (*Verbascum thapsus* L.) oils: The effectiveness of *V. thapsus* fruit, leaf and root oil extracts against pathogenic microbes was evaluated by MIC, MBC and MFC as indicated in Table 4. The root oil extract has exhibited strongest bactericidal activity with MIC (0.19 µL/mL) and corresponding MBC (0.25 µL/mL) against *S. aureus* while the weakest bactericidal activity with MIC (0.75 µL/mL, the largest value) and

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), Minimum Fungicidal Concentration (MFC) *Verbascum thapsus* fruit, leaf and root oils

Test pathogens	Oil extract	MIC (µL/mL)	MBC/MFC (µL/mL)
Escherichia coli	Fruit	0.75	1.50
	Leaf	0.75	1.25
	Root	0.38	0.50
Stapyhlococcus aureus	Fruit	0.50	0.88
	Leaf	0.31	0.50
	Root	0.19	0.25
Aspergillus versicolor	Fruit	0.25	0.50
	Leaf	0.63	1.25
	Root	0.38	0.38
A. niger	Fruit	0.09	0.18
	Leaf	0.38	0.75
	Root	0.25	0.38

corresponding MBC (1.50 µL/mL) was recorded for fruit oil against *E. coli* indicating that *S.aureus* is more susceptible to the oil extract than *E. coli* and also suggesting root oil possesses strongest antibacterial potential in common mullein (*V. thapsus* L.).

By contrast, fruit oil extracthas demonstrated strongest antifungal activity with MIC (0.09µl/ml, the least value) and MFC (0.18 µL/mL) against *A. niger* whereas the weakest antifungal activity with MIC (0.63 µL/mL) and the corresponding MFC (1.25 µL/mL) was observed for the leaf oil extract against *A. versiclor* showing that *A. niger* was more susceptible to the oil extract than *A. versicolor* and the fruit oil was more effective antifungal potential than root and leaf oils in *V. thapsus*. Similar study was conducted by Khan *et al*.[17] who evaluated methanol extract of aerial parts of *V. thapsus* against four gram-positive and five gram-negative bacteria.

CONCLUSION

The present study showed that soxhelt extracted oils from common mullein (*V. thapsus* L.) fruit, leaf and root are a good source of antioxidant and antimicrobial activities. The root oil extract was found to be the best for antibacterial activities, whereas the fruit oil extract was found to be the best for antifungal activities. The whole plant part of *V. thapsus* can provide medicinal, economic and health benefit if utilized in food products. The biological activities of the plant should have to prove ethnobotanical uses of the plant. The efficiency of various sources of the extracts should have to be assessed for better use of *V. thapsus* in pharmaceutical and food industries. Hence, suggesting possible exploitation of these plants for their antimicrobial active principles for the development of novel herbal-based antimicrobials.

Authors' contribution: Zekeria Yusuf: initiation and design of the study, Lab experiment, data analysis; Giorgis Hailu: Lab experiment, data collection and write up of the document; Mulugeta Desta: Analysis and interpretation of data. All authors contributed to drafting the article and revising it critically for important intellectual content.

ACKNOWLEDGMENTS

The authors are grateful to Haramaya University Research Office for their financial support and Laboratory facility.

Funding: This project was funded by Haramaya University Research grant, under project code: HUIF_2019_06_01_96.

Availability of data and materials: The data materials will be available on request.

Ethics approval and consent to participate: The ethical approval is not applicable for this manuscript since it has no animal experiment according to Haramaya University's ethical committee.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

01. Karaveliogullari, F.A. and Z. Aytac, 2008. Revision of the genus Verbascum L.(Group A) in Turkey. Bot. Res. J., 1: 9-32.
02. Turker, A.U. and N.D. Camper, 2002. Biological activity of common mullein, a medicinal plant. J. Ethopharmacol., 82: 117-125.
03. Prakash, V., S. Rana and A. Sagar, 2016. Studies on antibacterial activity of *Verbascum thapsus*. J Med. Plants Stud., 4: 101-104.
04. Hartleb, I. and K. Seifert, 1994. Songarosaponin D-A triterpenoid saponin from Verbascum songaricum. Phytochemistry, 35: 1009-1011.
05. Meurer-Grimes, B., D.L. McBeth, B. Hallihan and S. Delph, 1996. Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. Int. J. pharmacogn., Vol. 34, 1076/phbi.34.4.243.13220
06. Magiatis, P., D. Spanakis, S. Mitaku, E. Tsitsa, A. Mentis and C. Harvala, 2001. Verbalactone, a New Macrocyclic Dimer Lactone from the Roots of *Verbascum undulatum* with Antibacterial Activity. J. Nat. prod., 64: 1093-1094.
07. Hamayun, M., S. Afzal and M.A. Khan, 2006. Ethnopharmacology, indigenous collection and preservation techniques of some frequently used medicinal plants of Utror and Gabral, district Swat, Pakistan. Afr. J. Traditional Complementary Altern. Med., 3: 57-73.

08. AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., pp: 200-210.
09. AOAC, 2000. Official Methods of Analysis of the AOAC International (2000). 7th Edn., AOAC International, Gaithersburg, Maryland, USA..
10. Clinical and Laboratory Standards Institute (CLSI), 2015. Methods for Antimicrobial Dilution and Disk Susceptibility of Infrequently Isolated or Fastidious Bacteria. 3rd Edn., Clinical and Laboratory Standards Institute, Wayne, USA., Pages: 98.
11. Andrews, J.M., 2012. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother., 48: 5-16.
12. Morshed, H., S. Md, P. Salma, A. Maizbha Uddin, M.S. Islam and A.G.M.M.S. Mostofaand, 2012. Antimicrobial and cytotoxic activity of the methanol extract of *Paederia foetida* linn. (Rubiaceae). J. Appli. Pharmace. Sci., 2: 77-80.
13. SAS Institute, 2011. SAS enterprise guide, Version 9.2. SAS Inst., Cary, NC, USA.
14. Abdulkadir, A.G. and W.L.O. Jimoh, 2013. Comparative analysis of physico-chemical properties of extracted and collected palm oil and tallow. ChemSearch J. 4: 44-54.
15. Dulger, B., S. Kirmizi, H. Arslan and G. Guleryuz, 2002. Antimicrobial activity of three endemic Verbascum species. Pharma. Biol., 40: 587-589.
16. Dulger, B. and G. Dulger, 2018. Antibacterial activity of verbascum antinori. Konuralp Med. J., 10: 395-398.
17. Khan, A.M., R.A. Qureshi, S.A. Gilani and F. Ullah, 2011. Antimicrobial activity of selected medicinal plants of Margalla hills, Islamabad, Pakistan. J. Med. Plants Res., 5: 4665-4670.