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BIOACTIVE COMPOUNDS PROFILING IN *Rumex vesicarius* AND *Terminalia catappa* PLANTS USING GC-MS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants constitute an innumerable number of valuable therapeutic metabolites. Their easy availability and lesser side effects, make them a suitable choice for bioactive compounds against various infectious diseases. This study highlights the phytochemical profiles of *Terminalia catappa* extract and *Rumex vesicarius* whole plant extract. Extracts of the plant prepared using soxhlet and preliminary phytochemical analysis done following standard chemical methods. The identification of the phytochemical was further done using FTIR and GC-MS techniques. The results indicate the presence of a wide range of phytochemical constituents in ethanolic, methanolic, aqueous, hexane, and acetone extracts of *Terminalia catappa* and *Rumex vesicarius* plant.

Keywords: Bioactive compounds; chromatogram; phytochemicals; retention time; soxhlet extraction.

1. INTRODUCTION

In present scenario, the constant change in lifestyle and environment has led to the emergence of various diseases and medical conditions, for which treatments are yet to be discovered. The main challenges researchers face for the chemical and synthetic drugs are high cost and side effects. The medicinal plants are one of the viable alternatives for the treatment with less side effects. The emergence of natural resources as a rich source of drugs against a variety of diseases is a point of interest for researchers owing to their low cost and unsubstantial side effects. The World Health Organization has confirmed the importance of traditional medicine to a majority of the world's population and encourages all countries to preserve and to use the safe and positive elements of traditional medicine in their national health systems [1]. Both the proposed plants i.e, *Rumex vesicarius* and *Terminalia catappa* have shown antidiabetic activity in some in-vitro assays, but their exact mechanism as well as the essential phytoconstituents are still unexplored extensively. This study is further planned to decipher the mechanism behind the

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activity of the phytoconstituent responsible for the antidiabetic activity of both the plants.

Rumex vesicarius L., also known as Hammeidda [2] belongs to the family Polygonaceae. The plant is a annual shrub and is majorly indigenous to northern Africa and Asia (Pakistan and India) [3]. It has a rich source of Beta carotene, [4], vitamins C, proteins, lipids and organic acids, minerals (K, Na, Ca, Mg, Fe, Mn and Cu) [5-8]. Its medicinal properties have been well explored and action were reported as good aphrodisiac agent [9], reduce biliary disorders, control cholesterol [10]. Disorders like hepatic diseases, bad digestion, constipation, calcules, heart troubles, pains, diseases of the spleen, hiccough, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea are reported to have been treated using this plant in any form. *Terminalia catappa*, family Combretaceaeis widely distributed in tropical and subtropical climate countries, as salinity and winds are its suitable environment. It is used in the treatment of dermatitis, hepatitis, diarrhoea and pyresis [11, 12]. In Caribbean it is used against gastritis and urinary infection. Earlier work has been reported which explore antimicrobial, antifungal, antioxidant, antimetastatic, anti-inflammatory, mutagenic, aphrodisiac and antidiabetic properties of this plant leaves, roots and stem.

A wide range of active constituents from plants have been reported earlier through various studies. These active constituents are majorly phytochemicals produced by plants which have beneficial properties like antibacterial, antifungal, antioxidant, anticancer and many more [13]. Only small proportions of such compounds are known and have made their way to market in the form of drugs or some products. Due to the health promoting potential of selected medicinal plant species i.e, *Rumex vesicarius* and *Terminalia catappa*, nutritional characterization and chemical composition was carried out in this study. This work also aims to investigate these two plant species for their active components and analyze them as potential agents against diabetes mellitus which is a very common health problem in the general population. Further research in this field will allow for the prediction and the development of a peptide profile from a natural extract with a certain biological function for use in diabetes prevention or therapy.

2. MATERIALS AND METHODS

The step-wise method adopted for this research to detect the presence of various bioactive compounds in plant *Rumex vasicarius* and *Terminalia catappa* plants are mentioned below.

2.1 Experimental Materials

2.1.1 Collection and authentication of plant materials

The experimental materials used for this work were whole plant sample of *Terminalia catappa* and *Rumex vesicarius.* These plants were collected from Wazirabad garden of New-Delhi and local nuseries of Lucknow respectively. Plant samples were authenticated by Dr. L.B. Chaudhary, senior principal scientist and curator of herbarium plant diversity, systematics and herbarium division, CSIR-NBRI, Lucknow, Uttar Pradesh, India. Voucher specimens deposited in the CSIR-NBRI herbarium (LWG), Lucknow for future reference (Accession No:-108224, 108275).

2.1.2 Extraction

Extraction was done using method described in the earlier work by Suryavanshi et al. [13]. Briefly, the whole plant samples of *Rumex vesicarius* and *Terminalia catappa* were thoroughly washed and dried in the shade. These plant samples were properly pulverized, and the coarsely powdered plant samples were exposed to successive extraction using solvents-ethanol, acetone, and ethyl acetate by the continuous hot perculation method at 70˚C in the Soxhlet apparatus.

2.1.3 Functional Group Analysis using FTIR

It is the most common type of technique used to identify the functional groups possessed by each chemical compounds thus helping in identification. The instrument is based on the fact that each compound absorbs certain wavelength of light which are characteristics for functional bonds. It helps in identification based on the annotations available with the peaks of the spectrum. The extracts prepared in earlier section were dried, divided into two segments and stored for analysis of GC-MS and FTIR. The FTIR portion powder was dissolved and loaded in FT-IR spectroscope (Bruker Tensor 27 IR), with a Scan range from 400 to 4000 cm-1 with a resolution of 4 cm-1.

2.1.4 Active component Analysis using GC-MS

Determination of active component profiles in the extracts of *Terminalia catappa* and samples of *Rumex vesicarius* were analysed using GC-MS Analysis equipment (Thermo Scientific Co.) It helps to detect different substances present within the test sample.

Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II.
The experimental conditions for GC-MS system-
Column: TR 5-MS capillary standard polar
Dimension: 30Mts, ID: 0.25 mm
Film thickness: 0.25µm.
Carrier gas: He
Flow rate of mobile phase: 5.0 ml/min.
Temperature programme (oven temperature): 40°C raised to 350°C at 5°C/min
Injection volume: 1 µl.
Solvent for samples dissolving: Methanol Range: 50-650 m/z

The probable matched compounds were identified by comparing by using Wiley Spectral library search programme.

3. RESULTS AND DISCUSSION

The six extracts designated as RVA (*Rumex vesicarius* acetone), RVE (*Rumex vesicarius* ethanol), RVEA (*Rumex vesicarius* ethyl acetate), TCA (*Terminalia catappa* acetone), TCE (*Terminalia catappa* ethanol) and TCEA *Terminalia catappa* ethyl acetate) from sample of *Rumex vesicarius* and samples of *Terminalia catappa* respectively. The soxhlet extracts were first tested for their phytochemicals and showed presence of flavonoid in methanolic, acetone, aqueous and n-hexane in *Terminalia catappa* [13] and glycosides present in ethanolic and methanolic extracts of *Rumex vesicarius* [13]. The extracts which showed preliminary phytochemical tests positive, were further identified using GC-MS analysis and the functional group identification done using FTIR. The FTIR spectrum is shown in the Fig. 1 and the identified class of compounds present in different extract of both selected plants is compared in the Table 1.

In the interpretations it was observed for *Rumex vasicarius* that in all three extracts viz. n –Hexane, Aqueous, and Methanol Carboxylic acid were present, the other functional group reported were found to be Amine Salts, Alkanes, Alcohol (Primary and Secondary). While the exceptional compounds class found in n–Hexane extract were Sulfoxide, Anhydride; Aqueous extract consisted Aliphatic ether; Methanolic extract constituted Sulfonyl Chloride, Aldehydes and Aliphatic ether. In the extracts of *Terminalia catappa* Conjugated Aldehydes, Carboxylic Compounds, Amine compounds and Amine salts were found to be common; the exceptional compounds in n–Hexane were found to be Vinyl Ether; In Methanol Aromatic esters were estimated.

Table 1. Table showing the comparison of the classes of compounds present in different solvent extracts of *Rumex vesicarius* and *Terminalia catappa*

Solvent used for extraction	Compound class	
	Rumex vesicarius	***Terminalia catappa***
n-Hexane	Carboxylic acid	Conjugated aldehyde
	Amine salt	Amine
	Alkane (methylene group)	Alkane
	Aldehyde	Vinyl ether
	Sulfoxide	Aliphatic ether
	Anhydride	Alkene
	Alkene	-
Aqueous	Carboxylic acid	Alcohol
	Amine salt	Aromatic compounds
	Aldehyde	-
	Alkane	-
	Primary alcohol	-
	Aliphatic ether	-
Methanol	Carboxylic acid	Primary amines
	Conjugated aldehyde	Amine salts
	Tertiary amide	Amine salts
	Sulfonyl Chloride	Carboxylic Acid
	Amine	Aromatic Ester
	Aliphatic ether	Amine
	Primary Alcohol	-

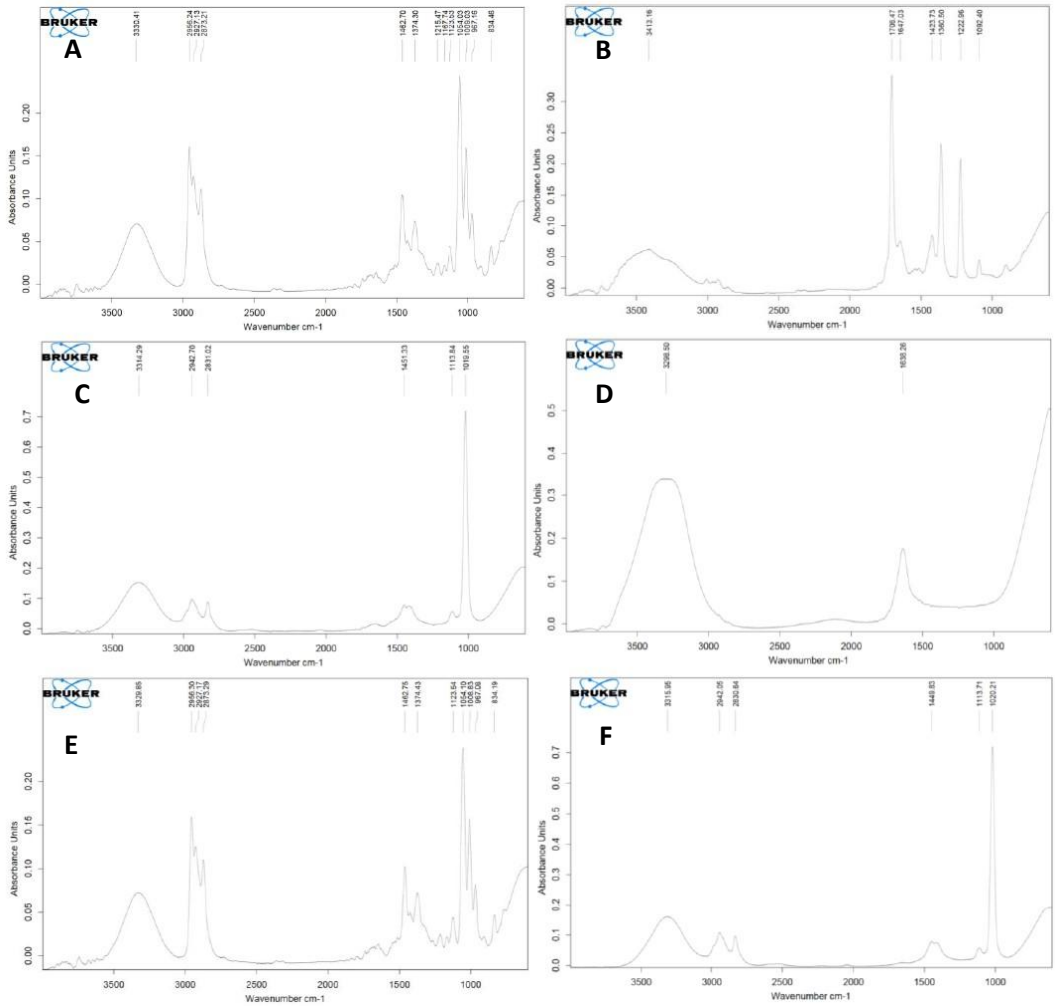

Fig. 1. FTIR Spectrum [A] *n- Hexane* extract of *Rumex vesicarius* [B] aqueous extract of *Rumex vesicarius* [C] Methanolic extract of *Rumex vesicarius* [D] aqueous extract of *Terminalia catappa* [E] n – Hexane extracts *Terminalia catappa* [F] methanolic extracts of *Terminalia catappa*

The lists of compounds identified by GC-MS analysis are summarized in the Table 2. The 9 peaks observed in case of RVA extract were Undecane (15.58 min), Eicosane 2-methyl- (36.81 min), 2,4-Di-tert-butylphenol (37.81 min), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (45.52 min), Methyl tetradecanoate (46.11 min), Hexadecanoic acid, methyl ester (53.04 min), 7,10-Octadecadienoic acid, methyl ester(58.28 min), Methyl stearate (59.29 min), Octadecane, 3-ethyl-5-(2-ethylbutyl) (59.55 min). The identified compounds matched with the library and highest probability score are mentioned along with their retention time.

Other two extracts of Rumex vesicarius i.e., RVE and RVEA showed 7 and 11 peaks respectively. Compounds matched were 1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl- (45.12 min), Cyclopentanetridecanoic acid, methyl ester (46.07 min), Hexadecanoic acid, methyl ester (53 min), enzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (53.52 min), Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3à,5à)- (58.24 min), 10-Octadecenoic acid, methyl ester (58.43 min) and Methyl stearate (59.25 min) for RVE while RVEA extract chromatograms identified peaks of Tramadol (10.6 min), Octadecane, 1,1'-[1,3-propanediylbis(oxy)] bis- (11.25 min), Octasiloxane (11.76 min), Heptasiloxane (15.64 min), Hexasiloxane (15.65 min), Cyclohexasiloxane, dodecamethyl- (15.66 min), (5á)Pregnane-3,20á-diol (15.67 min), 2-Trimethylsiloxy-6-hexadecenoic acid, methyl (15.68 min), Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl- (17.66 min), (5á)Pregnane-3,20á-diol (18.26 min) and Methyl 14-methyl-eicosanoate (59.37 min).

Table 2. Summarized Table showing the comparison of the identified compounds present in different extracts of *Rumex vesicarius* and *Terminalia catappa*

Solvent used for extraction	Compound Name	
	Rumex vesicarius	***Terminalia catappa***
Acetone	Undecane	Aniline
	Eicosane, 2-methyl-	Silanediamine, 1,1-dimethyl-N,N'-diphenyl-
	2,4-Di-tert-butylphenol	Silanediamine, 1,1-dimethyl-N,N'-diphenyl-
	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Galactonicphenylhydrazide
	Methyl tetradecanoate	Propanal, 2,3-dioxo-3-(3-pyridyl)-, 1,2-bis(phenylhydrazone)
	Hexadecanoic acid, methyl ester	Hexasiloxane
	7,10-Octadecadienoic acid, methyl ester	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4 -diyl)]-, diacetate
	Methyl stearate	Decanoic acid, 10-bromo-, methyl ester
	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Methyl glycocholate, 3TMS derivative
Ethanol	1,3-Dioxane, 5-(hexadecyloxy)-2-pentadecyl-,	Undecane
	Cyclopentanetridecanoic acid, methyl ester	Octasiloxane
	Hexadecanoic acid, methyl ester	Cycloheptasiloxane, tetradecamethyl-
	enzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	Heptasiloxane,
	Androstane-11,17-dione, 3 [(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3à,5à)-	á-D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-2,4,6-trioxo-5-pyrimidinyl)- 1,1-dimethylpropyl 2,3,4-tris-O-(trimethylsilyl)-,
	10-Octadecenoic acid, methyl ester	Hexadecanoic acid, methyl ester
	Methyl stearate	Methyl stearate
	-	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-
Ethyl acetate	Tramadol	1-Pentanol
	Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	1-Pentanol
	Octasiloxane,	1-Pentanol
	Heptasiloxane	Butanoic acid, 3-methyl-, 3-methylbutyl ester
	Hexasiloxane	Butane, 1,1'-[methylenebis(oxy)]bis[3-methyl-
	Cyclohexasiloxane, dodecamethyl-	Butane, 1,1'-[ethylidenebis(oxy)]bis[2-methyl-
	(5á)Pregnane-3,20á-diol	Butane, 1,1'-[ethylidenebis(oxy)]bis[2-methyl-
	2-Trimethylsiloxy-6-hexadecenoic acid, methyl	diethyl 2-hydroxy-3-(tetrahydrofuran-2-yl)succinate 2-hydroxy-3-(tetrahydrofuran-2-yl)succinate
	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-	diethyl 2-hydroxy-3-(tetrahydrofuran-2-yl) succinate
	(5á)Pregnane-3,20á-diol	-
	Methyl 14-methyl-eicosanoate	-

Ethanolic, aqueous and acetone extracts from sample of *Terminalia catappa* showed the presence of the varied number of compounds as condensed in table 2.The probably matched compounds identified by comparing by using Wiley Spectral library search program are given for each extract. TCA extract showed presence of Aniline (10.21 min), Silanediamine, 1,1-dimethyl-N,N'-diphenyl- (10.49 min), Silanediamine, 1,1-dimethyl-N,N'-diphenyl- (11.04 min), Galactonicphenylhydrazide (13.12 min), Propanal, 2,3-dioxo-3-(3-pyridyl)-, 1,2-bis(phenylhydrazone) (13.84 min), Hexasiloxane (37.52 min), (5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4 -diyl)]-, diacetate (45.63 min), Decanoic+ acid, 10-bromo-, methyl ester (52.99 min), Methyl glycocholate, 3TMS derivative (59.24 min).

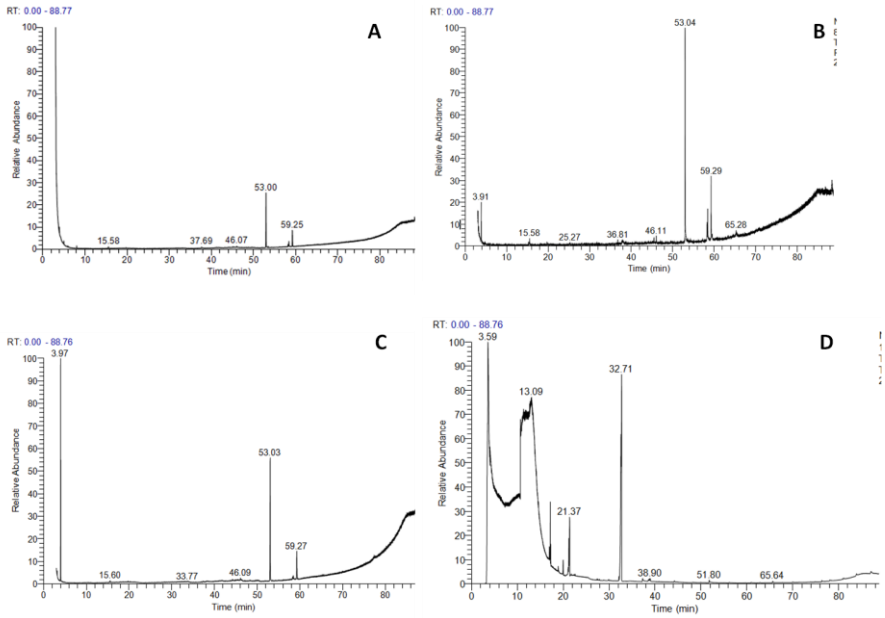

Fig. 2. GC-MS Chromatogram of *Rumex vesicarius* and *Terminalia catappa* plant extracts [A] RV [B] RVE [C] TCE [D] TCEA

TCE shows Undecane (15.6 min), Octasiloxane (19.78 min), Cycloheptasiloxane, tetradecamethyl- (37.25 min) Heptasiloxane (44.18 min), á-D-Glucopyranosiduronic acid, 3-(5-ethylhexahydro-2,4,6-trioxo-5-pyrimidinyl)- 1,1-dimethylpropyl 2,3,4-tris-O-(trimethylsilyl)- (46.1 min), Hexadecanoic acid, methyl ester (53.03 min), Methyl stearate (59.27 min), Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- (58.45 min) and TCEA extract had 1-Pentanol (10.68 min), 1-Pentanol (12.87 min), 1-Pentanol (13.06 min), Butanoic acid, 3 methyl-, 3-methylbutyl ester (17.17 min), Butane, 1,1'-[methylenebis(oxy)]bis[3-methyl- (19.98 min), Butane, 1,1'-[ethylidenebis(oxy)]bis[2-methyl- (21.24 min), Butane, 1,1' [ethylidenebis(oxy)] bis [2-methyl- (21.37 min), diethyl 2 – hydroxyl -3-(tetrahydrofuran – 2-yl)succinate 2-hydroxy-3-(tetrahydrofuran-2-yl)succinate (32.59 min), diethyl 2-hydroxy-3-(tetrahydrofuran-2-yl)succinate (32.71 min). Therefore, these two plant extracts were observed to possess phytochemical compounds which have not been explored in earlier studies. This study aims to exert any effort in finding components that could have high medicinal value. As a result, this study could serve as a basis for a more focused search for bioactive chemicals produced by these understudied plant species.

4. CONCLUSION

The present study focuses on evaluating the active components in the plant of *Terminalia catappa* and *Rumex vesicarius.* These plants are known to use in medications for a long time. In order to assess the detailed profile of bioactive compounds in the selected medicinal plants, FTIR and GC-MS chromatography techniques were used. We aim to explore more bioactive compounds from their samples which could have wider applications to solve health-related issues in living beings. The phytochemical compounds of these plants were extracted using soxhlet apparatus in solvents with increasing polarity. The results suggest that the extraction solvent and analytic procedures used are effective for isolating and identifying natural chemicals from plant samples. The extract of plant *Rumex vesicarius* RVA, RVE and RVEA showed presence of 9, 7 and 11 compounds respectively while the solvent extract of plant *Terminalia catappa* TCA, TCE and TCEA revealed the presence of 9, 8 and 9 peaks respectively for different compounds by GC-MS Chromatogram technique. Thus, this process led to the extraction of numerous compounds of the wide spectrum based on their solubility need. A study on phytochemical profiles of these plants helped to unearth more active compounds that could be studied exhaustively in the future to add to its already known and existing medicinal properties. The probably matched compounds identified by comparing by using Wiley Spectral library search program. The presence of essential and effective phytochemical compounds in these plant extracts provides an idea of considering these plant extracts for evaluation of their biological activity. This will also help to obtain a comparative outcome of these extract with other extract, already detected for such activities. The knowledge about the

presence of the certain category of the phytochemical compounds help to elucidate the application of these extracts and also makes the further evaluation more selective and specific. Overall, findings of this study shed light on the medicinal characteristics of these species and suggest that *Rumex vesicarius* and *Terminalia catappa* species could be used as sources of bioactive chemicals for further research analysis and application in the pharmaceutical industry.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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