Qualitative Phytochemical Screening of *Merremia dissecta* (Sepals & Fruit)

Jawad Ahmad, Abu Bakar Ali, Hazrat Bilal*

Department of Chemistry, Government Post Graduate College Dargai, District Malakand, Khyber Pakhtunkhwa, Pakistan

Abstract

Medicinal plants are widely used for medicinal purposes in pharmaceutical industries due to the presence of various phytoconstituents. This research is based on the phytochemical study of *Merremia dissecta* (fruits and sepals) in ethanol. Primary phytochemicals such as carbohydrates, reducing sugar, fixed oils and lipids, starch, hexose sugar, amino acids, and secondary phytochemicals such as betacyanin, cardiac glycoside, cyanogenic glycoside, flavonoids, saponins, tannins, and triterpenoids were present in all ethanolic extracts of *Merremia Dissecta*. So present work revealed that ethanolic extracts of *Merremia dissecta* is the rich source of various primary and secondary phytochemicals and are a medicinally significant plant.

Keywords: *Merremia dissecta,* betacyanin, flavonoids, tannins, triterpenoids, saponins, phytochemical screening.

Corresponding author: Mr. Hazrat Bilal; *Department of Chemistry, Government Post Graduate College Dargai, District Malakand, Khyber Pakhtunkhwa, Pakistan; E-mail: hazratbilal279@gmail.com*

1. Introduction

The information about chemical components of the plant are required and very important, not just for the innovation of medicinal causes, but for such financial resources as oils and tannins etc, this knowledge can be used in opening new sources which are the precursors for the complex chemical compounds synthesis [1]. On the bases of the world health organization, 80% of people are dependent on old-style treatments which include the use of plant extracts or the active compounds present in plants. Plant parts remain the leading source of pharmaceutical drugs and activators used in common medicines [2]. The present-day medicament itself still depends mainly on the secondary phytochemicals of the plants [3]. Medicinal plants are considered generous and abundant assets of the chemical constituents which can be used in medicine development including pharmacologically, non-pharmacologically, or synthetic medicines. Due to the birth of life-threatening diseases, an approach to modern therapies has continued to develop. Scientific researchers all over the world are concentrating their attention on naturally extracted bioactive chemical compounds as they are considered to have minimum toxic side impacts [4]. The plants synthesize naturally occurring bioactive secondary metabolites which are of key importance pharmaceutically and are being examined for their anticancer and antioxidant activities leading to the development of new effective drugs [5]. Antioxidants remarkably and effectively protect cellular entities such as DNA, protiens, and lipids from oxidative damage [6]. The antioxidant capacity of plants has achieved great interest and concentration due to an increase in oxidative stress which has been marked as a main causal agent in the enhancement and evolvement of neurodegenerative and cardiovascular diseases [7]. Besides the antioxidant potentials, chemical compounds of plants have very effective and key use in the therapy of cancer which is obvious from the history of the f treatment of cancer since ancient times [8]. Of the estimated, about 250,000 plant species, just 6% are searched for biological activity and about 15% for phytochemical activity [9With this estimation, plants provide a new opportunity for research studies and investigation new chemical compounds and thus plants are worth exploring [5]. The term phytochemistry means chemistry in plants, in its natural organic chemistry product, and biochemistry of plants are intimately related. Phytochemicals are a chemical compound that is extracted from plants. These constituents are categorized as primary and secondary phytochemicals. They are classified depending on their function in plant metabolism. The primary phytochemical of the plant includes carbohydrates, pyrimidines, amino acids, proteins and chlorophylls, purines of nucleic acids etc [10]. While the secondary phytochemicals include Terpenes (monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, terpenes, sesquiterpenes, tertraterpenes and polyterpenes), phenolics (coumarin, furano-coumarins, lignin, flavonoids, isoflavonoids and tannins), nitrogen-containing compounds (alkaloids, cyanogenic glucosides and non-protien amino acids) and sulfur-containing compounds (glutathione, glucosinolates, phytoalexins, thionins, defensins and allicin) [11].

2. Botanical Description

Merremia dissecta was discovered by Nicolaus Joseph yon Jacquin in the Caribbean between 1755 and 1759. In his book Observationum botanicarum published in 1767 he called this plant *Convolvulus dissectus*. Philip Miller in 1768 named this plant *Convolvulus palmatus*, having no idea of Jacquin's book, or not recognizing they were the same species [12]. William bartram in 1773 located the same in northern Florida [13, 14]. Both Jacquin and Bartram showed the species in (Fig. 1). These plants are mostly found in coastal areas (calcareous soils). The seeds of *Merremia Dissecta* are presented for sale in the United States, Australia and Taiwan [15]. It has been noted many times that *Merremia dissecta* is exotic in Australia [16]. (Barker, Barker et al. 2005). This Specie has been moving by humans for many eras both decisively and accidentally [15]. This specie was recorded in Asia, America, Western Australia, Malesia especially Java and Africa (1905), so Rendle called this specie *"cosmopolitan"* [17]. Species of *Merremia* in different zones are listed in Table 1.

Image of *Merremia dissecta*

Table 1: Species of *Merremia* in different zones

Zones	Americas	Madagascar & Africa	Pacific & Asia	Australia
Overall species	29	39	48	13
Unique specie	27	31	40	03
Infraspecific taxas	06	09	20	-

Convolvulaceae contains 1840 species and 56 genera, with international allotment, but mostly in tropical and subtropical zone [18].

Perennial climbers, it has herb like stems and lightly bushy having hairs. 07-10 cm long leaves petiolate with lightly hairs. Leaves are resembling to the palm of hand having 05-07 lobes which are glabrous on both side. Flowers are long pediculate and 02-flowered cymosely pedicels are usually thickened toward the calyx nearly 02.5 cm long flower stalk 05.5-07.5 cm long having hair on the bottom. The sepals are glabrous and elongated 02 cm long and 01.3 cm wide. Corolla is white or pink and the middle is dark purple usually 04 cm long. Stamen are unequal 15-18 mm long having thin filament style is 22mm long while stigma is bi-globular. Pistils are 02.4- 02.5 cm long having 02 mm long ovary cup shaped. Fruit capsular are present inside the calyx is 1.4 cm in diameter when get mature spontaneously get open to release 2 or 4 seeds [19].

3. Phytochemicals in *Merremia dissecta*

A wide range phytochemical such as fatty acids, heterocyclic compounds, lauric acid, aleamide, dibutyl phthalate, 1,2-benzenedicarboxylic acid, stigmasterol, alpha–Amyrin, Beta-Amyrin, Mandelic acid, cyclopentasiloxane and phloroglucinol from methanolic extract of callus of *Merremia dissecta* [20]. 40 different chemical compounds were detected from leaf sample and 26 phytochemicals were extracted frespectivelrom callus of *Merremia dissecta* [21]. The phytochemical investigation of *Merremia dissecta* leaves showed that fat, protein, carbohydrates, ash, fiber and dry matter. Their percentages are 0.57%, 24.25%, 22.74%, 5.69%, 10%, 28% and 94.31%. Five novel chemical compounds namely 3α-acyloxytropanes, merresectines: 3α-(4-methoxybenzoyloxy)nortropane, 3α-kurameroyloxytropane, 3α-nervogenoyloxytropane, 3α-[4-(β-D-glucopyranosyloxy)-3-methoxy-5-(3-methyl-2-butenyl)benzoyloxy]tropane (β-D-glucoside), 3α,6β-di-(4-methoxybenzoyloxy)tropane (merredissine) were separated from *Merremia dissecta* [22

4. Inhibition effect of *Merremia dissecta*

It was found that the aqueous extricates of the leaves of the *Merremia dissecta* shows inhibition activity towards gram negative bacteria pseudommnas aeruginosa and Gram positive staphylocous aureus with a rate of inhibition diameter 10mm and 7mm respectively. The same extract has also an inhibition effect against aspergillus oryzae with the percentage of inhibition of 26.56%, 29.68% and 39.06% it concentration of 1000, 2000, and 3000ppm respectively this inhibition percentage was noticed to be reached up to 27.5%, 30% and 45% against pencillium [23].

5. Antielastase β activity of *Merremia dissecta* fractions

Merremia dissecta shows elastolytic activity which was determined by using the substrate of the enzyme *β*-elastase. The substrate of the enzyme *β*-elastase elastase, elastin congo red (100μL) was dissolved in Tris-HCl having PH of 8 at a concentration of 5mg/ml. Then it was mixed with 100μL of cell- free culture supernatant acquired from p.aeruginosa ATCC27853 grown during 24h. This culture was present in LB media containing 5mg/ml of *Merremia dissecta* fractions respectively. This reaction mixture was incubated 9 at 37°C for 24h and centrifuged at 13,000 rpm for 10 minutes. The enzyme potential was measured from absorbance (495nm) of the supernatant [24].

6. Antimicrobial acitivty of *Merremia dissecta*

Merremia dissecta also shows antimicrobial activity against six different microbial strains. The antimicrobial activity was observed against four bacteria namely Bacillus, thuringiensis, Bacillus subtills, escherchia coli and pseudomonas putida. *Merremia dissecta* also shows antifungal activity against two fungal strains namely candida albicans and Aspergillus niger. Four different plant extricatesof *Merremia dissecta* were prepared in four different solvents (Aqeous, Methanol, Ethanol and petroleum Ether). These plants extracts were used for their antimicrobial activity using TTZ test (Tetrazolium Chloride test). It was observed that among all these extricates alcoholic extricates had maximum antimicrobial potential (Ridhi, Shikha et al. 2015).

7. Antibacterial and Antibiofilm activity of *Merremia dissecta*

Antibacterial activity is also noticed for *Merremia dissecta* extricates. The bacterium screened was a biofilm of phenotypic variant of bacteria pseudomonas aeruginosa ATCC27853. The diethyl ether extract of leaves of *Merremia dissecta* only permitted 20% biolfilm formation. This potential was correlated with a very important inhibition of bacterial growth s. aureus biomass which was also strongly minimised by all extracts of *Merremia dissecta* upto (71-100%). All these potentials were not dependent on growth of bacteria [24].

8. Materials & Methods

Samples of sepals and fruit of *Merremia dissecta* were collected from the region of Ghamgeen Abad, Ghari Usmani khel, tehsil Dargai, district Malakand Pakistan on 25th February 2021 (Fig.2). Mr. Rab Nawaz at department of botany Government Gostgraduate College Dargai, Malakand, Pakistan confirm identification of the plant. Fruits and sepals of the collected plant were cleaned with distilled water and kept for 03 weeks at room temperature to dried. Dried fruits and sepals were grinded into powder (Fig.3). 11g and 08g grinded powder of sepals and fruits were added to 200ml and 100ml of ethanol respectively. These solvents were placed in room temperature for one week for better extraction and by using Whatman no.42 filter paper the obtained extracts were filtered (Fig.4). After that filtered was placed in room temperature for some days to evaporate ethanol and get a viscous crude. Then some of the thick viscous crude form of ethanolic extracts were dissolved in ethanol and various phytochemical tests were performed.

Fig.2

Fig.3 Sepals and Fruits

Fig.4 Sepals and Fruits

9. Phytochemical screening

Using standard procedures (i-xiii) to performed the phytochemical screening for ethanol soluble fractions[25-31].

Primary Phytochemicals

i. Carbohydrate

Molish's test

02ml juice or peel extract, 01ml of Molish's reagent and only some drops of conc. H_2SO_4 were added. The creation of a purple or reddish ring shows the existence of carbohydrates.

Test for starch

A few ml sample in Aqueous extract and then 5mL 5% KOH solution ware added. A cinary colouration will appear for positive result.

ii. Reducing sugar

Benedict test

Parallel amount of the peel extract and Benedict's solution and for 05 mintes heated in a boiling water bath. The solution appears green, yellow, or red color depend on the quantity of reducing sugar existed in the solution.

Fehling test

Parallel amount of Fehling A and Fehling B reagents were mixed and 02ml of it was added to juice extract and boiled softly 10 to 15 minutes. First yellow and then brick-red precipitate seemed at the base of test tube which showed the existence of reducing sugars.

iii. Hexose sugar

Cobalt chloride test

03ml of peel extract and 02ml of cobalt chloride solution were mix up, then boiled it and then cooled. After that, added some drops of NaOH solution to it. The solution seems greenish blue (glucose) or purplish (fructose) or upper layer greenish-blue and lower layer purplish (a mixture of glucose and fructose).

iv. Amino acids

Xanthoproteic test

A few ml plant extract and then few drops of conc. HNO_3 ware add. Yellow colored solution for positive result.

Ninhydrin Test

The peel extract or sample juice is boiled with 02ml of 05% solution of Ninhydrin in boiling water bath for 10 minutes, violet color showed the positive result for amino acids.

v. Fixed Oils & Lipids

Filter paper test

A little amount of sample juice was individually squeezed between two filter papers & let to dry. Oil stain or grease mark formation on filter paper when seen below the direct sunlight specified the positive result of fixed oils.

Secondary Phytochemicals

vi. Betacyanin

Sodium hydroxide test

02ml of sample juice, 01ml of 02N NaOH was combined & for 05 minutes heated at 100°C. Creation of yellow color shows positive result for Betacyanin.

vii. Cardiac glycoside

The Acetic acid test

02ml peel extract ware added to glacial acetic acid having 01 drop of ferric chloride solution, with 01ml of conc. H_2SO_4 this will be under layered. Brown ring of generation shows deoxysugar Characteristics of cardenolides. A violet ring may generate slowly right through the thin layer.

viii. Cyanogenic glycosides

Sodium picrate test

First of all, filter paper was soaked in 10% picric acid solution and then in 10% sodium carbonate solution and then it was left for drying. Then the dried filter paper was entered to the corked beaker containing sample from the slit. The presence of halcones glycoside was indicated when the filter paper turns brick red or maroon color.

ix. Flavonoids

Alkaline reagent test

02ml of 02% NaOH solution was added to 1ml sample extract and then 02ml of 02% NaOH solution ware added. An intense yellow color appeared which showed the presence of flavonoids. The yellow color disappeared by the addition of few drops of dilute HCl.

Conc. H_2SO_4 test

A few ml of sample extract and conc. H2SO4 ware added. For the positive result an orange colour will appear.

x. Saponins

Foam test

About 05ml of the juice of sample was added to 20ml of chloroform and then this sample solution was boiled in water bath and filtered. 10ml of filtrate was mixed with 05ml of distilled water. Till a stable persistent froth formation this mixture was shaken vigorously. Then 03 drops of olive oil were added to this persistent froth solution. The formation of emulsion indicated the presence of Saponins.

xi. Triterpinoides

Salkowski's test

A few drops of conc. H2SO4 was added to a few ml sample filtrateand the presence of Triterpenoides was indiacted by the formation of Golden yellow layer at the bottom of test tube.

xii. Tannins

Braymer's test

01ml sample filtrate was mixed with 03ml of distilled water. Then 03 drops 10% Ferric chloride solution was added to this mixture. The formation of Blue-green color indicated the presence of Tannins.

xiii. Terpenoids

Chloroform test

05ml of each extract was mixed with 02ml of chloroform and then 03ml of concentrated H2SO4 was carefully added. A reddish-brown coloration of the interface was formed which indicated positive result for the presence of Terpenoids.

5. Results & discussion

Phytochemical screening

This research was performed on various parts of *Merremia Dissecta* juices and peels extract which reviled presence of the numerous medicinal active components. Phytochemical active compounds present in them

were qualitatively analyzed for peel extract and pulp juice separately and their results are presented in the tables 2.

Table 2@: Phytochemical analysis of *Merremia Dissecta*

Primary Phytochemicals			
Test Names		**Sepal**	**Fruit**
Carbohydrates	Molish's test	+	+
	Test for starch	+	+
Reducing sugars	Benedict test	+	+
	Fehling test	+	+
Hexose sugars	Cobalt Chloride test	+	+
Amino acids	Ninhydrin test	+	/
	Xanthoproteic test	/	+
Fixed Oils and Lipids	Filter paper test	+	+
Secondary Phytochemicals			
Betacyanins	Sodium hydroxide test	+	+
Cardiac glycoside	Acetic acid test	+	+
Cyanogenic glycosides	Sodium picrate test	+	+
Flavonoids	Alkaline reagent test	+	+
	Conc. H_2SO_4 test	+	+
Saponins	Foam test	+	+
Triterpinoides	Salkowiski's test	+	+
Tannins	Braymer's test	+	+
Terpenoids	Chlorofo-rm test	+	+

Keywords:
+ = Presence of compounds
- = Absence of compounds
/ = Not attempted

10. Discussion

Phytochemical screening is very important for the detection and isolation of new and important constituents. The crude ethanolic extract of fruit and sepals were taken for the phytochemical analysis and showed a variety of bioactive primary and secondary phytoconstituents. Ethanolic extracts of the, fruits and sepals were taken for the phytochemical screening. After performing careful phytochemical screening by using different tests through various laboratory reagents for different phytochemicals, tests for primary phytochemicals such as carbohydrates, reducing sugar, starch, hexose sugar, amino acids, fixed oils and lipids, and secondary phytochemicals such as Betacyanin, cardiac glycoside, cyanogenic glycosides, flavonoides, Saponins, tannins, and Triterpenoids were positive for the sepals and fruit of *Merremia dissecta*.

11. Conclusion

The information collected in this investigation specified that *Merremia dissecta's* ethanolic extract is more rich in phytochemical than its n-hexane extract. *Merremia dissecta* is a source of various metabolites and therefore recommended as phytopharmaceutically important which had also been supported by a number of scientific studies related to its composition. This shows that *Merremia dissecta* is a medicinal plant and can be used for commercial and medicinal uses. Phytochemical screening of *Merremia dissecta* and its activities such as Inhibition effect, Antielastase beta activity, antimicrobial activity, Antibacterial and Antibiofilm activity of *Merremia dissecta* shows that it is a true important medicinal plant and therefore deserves wider recognition and more study.

Acknowledgements

We would like to thanks Mr. Yousaf Khan, Mr. Muhammad Ayaz, Mr. Kashif Ali, Mr. Imad Ali and Mr. Usman Khan, Government Post Graduate College Dargai for their suppurate to accomplish this research.

References

1. Mojab, F., M. Kamalinejad, N. Ghaderi and H. R. J. I. J. o. P. R. Vahidipour (**2010**). "Phytochemical screening of some species of Iranian plants." (2): 77-82.
2. Kumar, N. S., N. J. J. o. P. Simon and Phytochemistry (**2016**). "In vitro antibacterial activity and phytochemical analysis of Gliricidia sepium (L.) leaf extracts." 5(2): 131.
3. Akharaiyi, F., B. Boboye and F. J. W. A. S. J. Adetuyi (**2012**). "Antibacterial, phytochemical and antioxidant activities of the leaf extracts of Gliricidia sepium and Spathodea campanulata." 16(4): 523-530.
4. Greenwell, M., P. J. I. j. o. p. s. Rahman and research (**2015**). "Medicinal plants: their use in anticancer treatment." 6(10): 4103.
5. Ang, A. M. G., M. M. Enot, G. J. D. Baltazar, C. V. Alinapon, E. O. Buncales, G. B. J. A. J. o. B. Barbosa and L. Sciences (**2019**). "Antioxidant and cytotoxic activity of the leaf ethanolic extracts of Tithonia diversofilia and Gliricidia sepium from Bukidnon, Philippines." **8**(1): 9.

6. Sati, S. C., S. Nitin, U. Rawat and O. J. R. J. o. P. Sati (**2010**). "Medicinal plants as a source of antioxidants." 4(4): 213-224.
7. Kasote, D. M., M. V. Hegde and S. S. J. B. Katyare (**2013**). "Mitochondrial dysfunction in psychiatric and neurological diseases: cause (s), consequence (s), and implications of antioxidant therapy." 39(4): 392-406.
8. Cragg, G. M., D. G. Kingston and D. J. Newman (**2005**). Anticancer agents from natural products, Taylor & Francis Boca Raton.
9. Fabricant, D. S. and N. R. J. E. h. p. Farnsworth (**2001**). "The value of plants used in traditional medicine for drug discovery." 109(suppl 1): 69-75.
10. Krishnaiah, D., R. Sarbatly, A. J. B. Bono and M. B. Reviews (**2007**). "Phytochemical antioxidants for health and medicine a move towards nature." 2(4): 97-104.
11. Jamwal, K., S. Bhattacharya, S. J. J. o. a. r. o. m. Puri and a. plants (**2018**). "Plant growth regulator mediated consequences of secondary metabolites in medicinal plants." 9: 26-38.
12. Miller, P. (**1768**). The Gardeners Dictionary: Containing the Best and Newest Methods of Cultivating and Improving the Kitchen, Fruit, Flower Garden, and Nursery; as Also for Performing the Practical Parts of Agriculture.
13. Bartram, W. and F. Harper (**1958**). The Travels of William Bartram. Naturalist's Edition. Edited with Commentary and an Annotated Index by Francis Harper.[With Plates, Including a Portrait, and Maps., Yale University Press.
14. Bartram, W. and F. J. T. o. t. A. P. S. Harper (**1943**). "Travels in Georgia, and Florida, 1773-74. A Report to Dr. John Fothergill." 33(2): 121-242.
15. Austin, D. F. J. E. B. (**2007**). "Merremia dissecta (Convolvulaceae): Condiment, medicine, ornamental, and weed—A review." 61(2): 109-120.
16. Barker, B., R. Barker, J. Jessop and H. Vonow (**2005**). "Census of South Australian vascular plants."
17. Staples, G. (**2010**). A checklist of Merremia (Convolvulaceae) in Australasia and the Pacific, Singapore Botanic Garden.
18. Baker, J. and A. J. F. o. t. A. Rendle (**1905**). "Convolvulaceae." 4(2): 62-206.
19. Al-Hawshabi, O. S. S. J. J. o. P. and B. Sciences (**2016**). "A new alien record for the flora of Yemen: Merremia dissecta (Jacq.) Hallier f.(Convolvulaceae)." 11(2): 01-03.
20. Joshi, R., R. Meena, V. J. J. o. P. Patni and Phytochemistry (**2018**). "Comparative phytochemical analysis of bioactive constituents present in in vitro and in vivo plant parts of Merremia aegyptia and Merremia dissecta." **7**(1): 679-684.
21. van Putten, P. v. L. J. A. v. L. (**1979**). "Mandelic acid and urinary tract infections." 45(4): 622-623.
22. Jenett-Siems, K., R. Weigl, A. Böhm, P. Mann, B. Tofern-Reblin, S. C. Ott, A. Ghomian, M. Kaloga, K. Siems and L. J. P. Witte (**2005**). "Chemotaxonomy of the pantropical genus Merremia (Convolvulaceae) based on the distribution of tropane alkaloids." 66(12): 1448-1464.
23. Jasim, S. N., N. M. Salih, R. J. Kadim, A. H. AL-Bayar and S. A. J. A. C. M. Abd-Abdlatif (**2018**). "Quantitative Assessment Of Chemical Composition, Antimicrobial Efficacy And Some Active Compounds Of Merremia Dissecta." 2(2): 01-05.
24. Luciardi, M. C., M. V. Pérez Hernández, N. Muruaga, A. Bardón, M. E. Arena, E. J. E.-B. C. Cartagena and A. Medicine (**2016**). "Volatiles from subtropical convolvulaceae that interfere with bacterial cell-to-cell communication as potential antipathogenic drugs." (**2016**).
25. Vishnoi, N. (**1979**). "Advanced practical organic chemistry."
26. Harborne, A. (**1998**). Phytochemical methods a guide to modern techniques of plant analysis, springer science & business media.
27. Roopashree, T., R. Dang, S. Rani and C. J. I. J. o. A. r. i. N. p. Narendra (**2008**). "Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis." 1(3): 20-28.

28. Walag, A. M. P., A. B. M. Cepeda, A. S. Galenzoga and S. M. J. J. I. J. B. Sambaan (**2017**). "Initial phytochemical screening of the different parts of Mansoa alliacea L.(Garlic Vine)." 11(3): 227-231.
29. Deb, K., A. Kaur, S. A. K. Ambwani and T. J. J. o. m. p. s. Ambwani (**2018**). "Preliminary phytochemical analyses of hydromethanolic leaf extract of Melia azedarach L." 6(3): 4-8.
30. Elzagheid, M. I. J. W. J. o. C. E. (**2018**). "Laboratory activities to introduce carbohydrates qualitative analysis to college students." 6(2): 82-86.
31. Shaikh, J. R. and M. J. I. J. o. C. S. Patil (**2020**). "Qualitative tests for preliminary phytochemical screening: An overview." 8(2): 603-608.