ETHANO-PHYTO-SPECTRAL ANALYSIS OF MERREMA TURPETUM OF ANANTAPUR DIVISION, INDIA

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Abstract
The present study was carried out to evaluate the ethano, phyto and spectral studies of the methonalic extract of Merrema turpethum(MEMT). The potency of ethanol extract of the plant showed rich antioxidant properties. From this study it can be concluded that the (MEMT) showed significant hepatoprotective and antioxidant action. Many investigated spices had high levels of phenolics and exhibited high antioxidant capacity. The TEAC values of the spices ranged from 1.76 to 346 lM trolox/100 g dw, from 7.34 to 2021 lM trolox/100 g dw, and 13.8 to 2133 lM trolox/100 g dw for ABTS_+, DPPH_ and FRAP, respectively. The total phenolic content, measured using a Folin–Ciocalteu assay, ranged from 0.07 to 15.2 mg of gallic acid equivalents (GAE)/100 g dw. A positive relationship between TEAC (ABTS_+ and FRAP) values and total phenolic content, measured by HPLC.

Keywords: Spectral studies, phyto and ethano studies, methonalic extract, hepatoprotective, antioxidant properties, Merremia turpethum, HPLC, Folin–Ciocalteu assay, trolox, gallic acid.

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INTRODUCTION

Anantapur district was shaped during the year 1882 has been divided from Bellary district. It is part of the southern most districts of Rayalaseema of Andhra Pradesh. The district extends over an area of 19.130 sq. Km (or) 19.13 lakh hectors. Geometrically the district is located between 13° 14’ and 15° 14’ north latitudes and 76° 47’ and 78° 26’ East longitudes. Merremia turpethum is an important medicinal plant, in Ayurvedic and Unani systems of medicine it in worthy usage, it is a true panacea, and is a vital ingredient in more than 135 ayurvedic and herbal-mineral formulations[1]. The rhizome is used as purgative herbs, herbs for therapeutic enema, antidote herbs and herbs eliminating toxins. The rhizome is taken internally to treat oedema, anaemia, ascites, haemorrhoids, cough, asthma, rheumatism, fever, anorexia, obesity, melancholia, constipation, dyspepsia, flatulence, paralysis, gout hepato-splenomegaly, hepatitis, intoxication and abdominal tumours [2]. Externally a paste of the rhizome is used to treat worm infestation, wounds, pruritus, vitiligo scorpion stings, snakebites, ulcers, and other skin disorders.

II. PHYTOCHEMICAL ANALYSIS

Phytochemical analysis was conducted using standard protocols Sofowora, 1993 and Trease and Evans., 1997)[3]. A brief account of the different tests conducted was as follows:

SCREENING TESTS FOR SECONDARY METABOLITES

Chemical tests for various phytoconstituents in the extracts were carried out as described below.

TEST FOR STEROIDS

A. SALKOWSKI TEST:
Few drops of concentrated sulphuric acid are added to the chloroform extract, shaken well and on standing lower layer turn red in colour[4].

B. LIEBERMANN BURCHARDS TEST:
In the chloroform solution of the extract, few drops of acetic anhydride are added and mixed well [5]. 1 ml of concentrated sulphuric acid is added from the sides of the test tubes, a reddish brown ring is established at the junction of the two layers.

TESTS FOR TRITERPINES:

A. SALKOWSKI TEST:
In the chloroform solution of the extract few drops of acetic anhydride are added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of the test tubes, a red ring indicates Triterpenes[6].
B. TSCHUGAJUR TEST:
Excess of acetyl chloride and pinch of Zinc chloride are added to the chloroform solution kept aside for the reaction to subside and warmed on water bath, Eosin red colour is produced[7,8].

III. TEST FOR SAPONINS
A. FOAM TEST
Small amount of extract is shaken with a little quantity of water, the foam produced persists for 10 min, and it confirms the presence of saponins[9,10].

B. HEAMOLYTIC TEST
2ml of 1.8% Sodium Chloride solution is taken in two test tubes, 2 ml of 1.1% extract to the other, 5 drops of blood is added to each tube and gently mixed with the contents. Hemolysis observed under the microscope in the tube containing the extract indicates the presence of Saponins.

TEST FOR STEROIDAL SAPONINS:
A. SALKOWSKI TEST:
Few drops of concentrated sulphuric acid are added to the chloroform extract, shaken and on standing lower layer turn red in colour [11].

B. LIEBERMANN BURCHARDS TEST
In the chloroform solution of the extract, few drops of acetic anhydride are added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of the test tubes, a reddish brown ring is established at the junction of the two layers.

TESTS FOR ALKALOIDS
A. Mayer’s Test: (solution of Potassium mercuric iodide solution)
The filtrate was treated with Mayer’s reagent, the formation of cream colour precipitate indicates presence of alkaloids.

B. DRAGENDROFF’S TEST (Solution of potassium Bismuth iodide)
The filtrate was treated with Dragendroff’s reagent. The formation of reddish brown colour precipitate indicates the presence of alkaloids [12].

C. WAGNER’S TEST (Solution of Potassium Iodide in Iodine)
The filtrate was treated with Wagner’s reagent. The formation of reddish brown color precipitate indicates the presence of alkaloids.
D. HAGER’S TEST (Solution of saturated picric acid) 
The filtrate was treated with Wagner’s reagent. The formation of yellow color precipitate indicates the presence of alkaloids.

IV. TESTS FOR CARBOHYDRATES
A. FEHLING’S TEST (Solution of copper sulphate, sodium tartarate and sodium hydroxide) The extract when heated with Fehling’s A & B solutions, gives an orange red precipitate, showing the presence of reducing sugar.

B. MOLISCH’S TEST (Solution of alpha- naphthol in alcohol) The extract is then treated with Molisch’s reagent and conc. Sulphuric acid is passed along the sides of the test tube, a reddish violet ring shows the presence of carbohydrates[13].

C. BENEDICT’S TEST (solution of copper sulphate, sodium citrate and sodium carbonate) The extract on heating with Benedict’s reagent, formation of brown precipitates indicates the presence of sugar.

D. BARFOED’S TEST
Barfoed’s reagent is added and boiled on water bath for few minutes[14]. Reddish precipitate is observed for the presence of carbohydrates.

V. TESTS FOR TANNINS:
A. FERRIC CHLORIDE TEST
With 1% ferric chloride solution, the extract gives blue, green or brownish green colour indicating the presence of tannins.

B. GELATIN TEST
When the extract is treated with 1% solution of gelatin containing 10% sodium chloride white precipitate is obtained.

TEST FOR FLAVONOIDS
A. SCHINODA TEST
The alcoholic solution with few fragments of magnesium ribbon and conc. HCl produces magenta colour after a few minutes.

B. FERRIC CHLORIDE TEST
Few drops of ferric chloride solution are added to a little quantity of the alcoholic extract. A blackish green colour produced indicates the presence of phenolic nucleus.
C. LEAD ACETATE TEST
Little amount of lead acetate solution (10%) is added to the alcoholic solution, yellow precipitate was observed indicating the presence of flavonoids.

D. ZINC HYDROCHLORIC ACID TEST
The alcoholic solution is treated with pinch of zinc dust and few drops of conc. Hydrochloric acid, magenta colour is produced after few minutes indicates the presence of flavonoids.

VI. TEST FOR GLYCOSIDES
A. BALJET TEST
To the extract, sodium picrate solution is added. It shows yellow to orange colour is observed indicates the presence of glycosides.

B. LEGAL TEST
The extract is dissolved in pyridine and sodium nitroprusside solution are in addition to make alkaline, pink or red colour is produced.

C. KELLER KILLIANI TEST
The extract was dissolved in glacial acetic acid, after cooling 2 drops of ferric chloride solution are added to it. These contents are transferred to a test tube containing 2 ml of conc. Sulphuric acid a reddish brown colour ring is noted at the junction of two layers.

VII. TEST OF ANTHARACYANINS AND ANTHOCYANIDINS
SODIUM HYDROXIDE TEST
2ml of Sample + 1.0ml of (2N) NaOH solution was added and heated on a water bath for few minutes. Formation of bluish green colour indicates the presence of Anthocyanin and formation of yellow colour indicates the presence of Betacyanin.

VIII. PROCESSING OF PLANT MATERIAL FOR SOLVENT EXTRACTION
EXTRACTION PROCESS FOR THE SELECTED PLANT MATERIALS
The selected plant material *Merremia turpethum* were subjected for the hot non-sequential extraction process by using soxhlet apparatus as shown in fig.1. The plant material was extracted using the different solvents (n-hexane, ethanol and hydro-alcohol system 1:1) by increasing in polarity [15].

PREPARATION OF EXTRACTS
The plant powders were subjected to serial solvent extraction and extracts n-hexane, ethanol, hydroalcohol were prepared respectively. These extracts were further concentrated by using Buchi
(R-200) Rotavapour. The concentrated semi-solid extracts were stored in air tight screw cap vials and kept in refrigerator till further use (Mohapatra et al., 2010)[16].

PREPARATION OF VARIOUS EXTRACTS OF MT

Leaves were shade dried and they were sent for processing. The processing of the 1kg powder material of each plants was subjected for non-sequential extraction as discussed earlier.

RESULTS AND DISCUSSIONS

SPECTRAL CHARACTERISATION OF ISOLATED COMPOUND

\[ ^1 \text{HNMR} - 7.19(\text{OH}), 4.61, 4.5(2\text{H, dd at H29}), 3.97-3.10 (\text{dd, 1H at H3}), 2.37 (\text{m, 1H, at H19}), 2.31 (\text{ m, 1H at H21}), 2.12 (\text{t, 1H at H15}), 2.10 (\text{ s, 3H, H30}), 1.61, 1.44,1.31, 1.24, 1.18, 1.12, \]

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1.09, 0.97, 0.95, 0.91, 0.75, 0.73, 0.71, 0.68 tertiary methyl protons are present in seven in number as shown in below figure2.

![Fig.2: ¹HNMR Spectrum of Compound 1](image)

**CONCLUSION**

The Hydro alcoholic extract of *Merremia turpethum* showed the presence of phytoconstitution similar to that of the ethanolic extract except amino acids. The isolated and purified compound was collected and subjected for the spectral studies on IR, ¹HNMR and MASS spectroscopy. From the data’s collected were compared with the existing data’s to conform that compound 1 (Lupeol) of *Merremia turpethum*.

**REFERENCES**


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