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Isolation, Phytochemical Screening and Pharmacological Evaluation of Tagetes erecta Leaves Extract

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Abstract— *Tagetes erecta plant Synonym Aztec Marigold. The Tagetes erecta commonly known as Genduphul. the leaves of plant collected and authenticated and powdered and extracted with methanol as solvent by soxhlet extraction method. The phytochemical evaluation and phytochemical screening of extract was done, the plant showed presence of flavanoids, tannin, alkaloids, carbohydrate, terpenoids. These constituent was separated from each other by thin layer chromatography, Isolated by column chromatography these spots was send for spectral analysis .the antiulcer activity was performed, by ethanol induced method*.

Keywords— *Tagetes erecta, flavanoid, Antiulcer*.

I. INTRODUCTION

Tagetes erecta family- Asteraceae Synonym Aztec marigold. The Tagetes genus contain 50 species of perennial herbaceous plant. Tagetes erecta plant commonly known as Genda phul. These are found in tropical and subtropical region such as India and Bangladesh and America The main chemical constituent present in Tagetes erecta are quercetagetin, flavanoids, triterpenoid, thiophene, syringic acid, methyl-3,5-dihyroxy-4-methoxy biotin, thienyl and ethyl gallate. The Tagetes erecta flowers is used as antioxidant anti-inflammatory antihyperlipidemic and antibacterial and hepatoprotective and anticancer, antimicrobial

II. MATERIAL AND METHODS

Extract used

Methanolic exract of leaves of Tagetes erecta

Experimental

Collection of plant -The fresh leaves of Tagetes erecta were collected from local region Authentication of plant - Authentication of leaves of Tagetes erecta were done at willingdon college .Distillation of solvent-Distillation of solvent done before extraction of plant material.

Method of extraction

Soxhlet extraction

Preparation of Extract -The collected leaves of plant were washed with water and dried under shade, after drying of plant material it was powdered by mixer grinder. The 100gm of powder extracted with methanol in soxhlet extractor at temperature of 40 degree C. The extraction was continued until solvent in the thimble become clear. These extract was stored in dessicator and used for further phytochemical study. The preliminary phytochemical study was performed.

Preliminary phytochemical investigation-

CHEMICAL TEST	RESULT
1. Test for glycosides	+
Test for Saponins	+
2. Test for Carbohydrate's	+
3. Test for Flavonoid's	+
4. Test for Alkaloid's	+
5. Test for tannin's	+

Physiochemical evaluation of extract was performed by different method such as the total ash value and acid insoluble ash value, water soluble ash value and total moisture content. this method was used to remove the adulteration from the extract.

Separation and Isolation: the methanolic extract of Tagetes erecta was separated by thin layer chromatography, the solvent system consist of Pet ether: Toulene: Methanol: Isopropyl alcohol (1:2:0.3:3 drops).this solvent system gives clear and distinct spots. The column chromatography was used for separation of these spots, consist of mobile phase, Pet ether: Toulene: Methanol: Isopropyl alcohol (1:2:0.3:3drops).the spots which was separated from column, analysed by infrared spectroscopy, nuclear magnetic resonance, mass spectroscopy.

Identification of Compound

Physical properties
Solubility- Methanol,Chloroform
Colour -Green
Melting point- 212^0C
Chemical Properties
Shinoda test
Lead acetate test

Pharmacological evaluation

Preparation of Plant Extract

The methanolic extract of Tagetes erecta plant was prepared in different concentration of 200 and 400ml/kg of body weight

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Ethanol induced gastric ulcer

The ulcer was induced by administrating ethanol. All the animals were fasted for 24 hrs before administration of ethanol. The albino rats of either sex were divided into five groups, each consisting of four rats. Groups are follows

Normal group	Vehicle
Control group	Ethanol(1ml/200kg)
Higher group(400mg/kg)	Methanolic extract of T. erecta
Lower group (200mg/kg)	Methanolic extract of T .erecta
Standard group	Ranitidine(50mg/kg)

The animals were anaesthetized 1hrs later with anesthetic ether and stomach was incised along with greater curvature and ulceration will be scored.

Determination of Ulcer index

Ulcer index (UI) was measured by using following formula:

$$UI = UN + US + UP \text{ X } 10{-}1$$

Where,
UI = Ulcer Index,
UN = Average number of ulcers per animal,
US = Average number of severity score,
UP = Percentage of animals with ulcers

Determination of Total acidity(5,6,7)

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and, titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following formula

Acidity = Vol. of NaOH × N × 100 mEq/L/0.1

Percentage Decrease in Acidity = Acidity of Control - Acidity of Test /Acidity of Control

Determination of Free acidity

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

Gastric volume

Formula –

% Decrease in Gastric volume = Gastric volume of Control - Gastric volume of Test /Gastric volume of control X100

Determination of pH

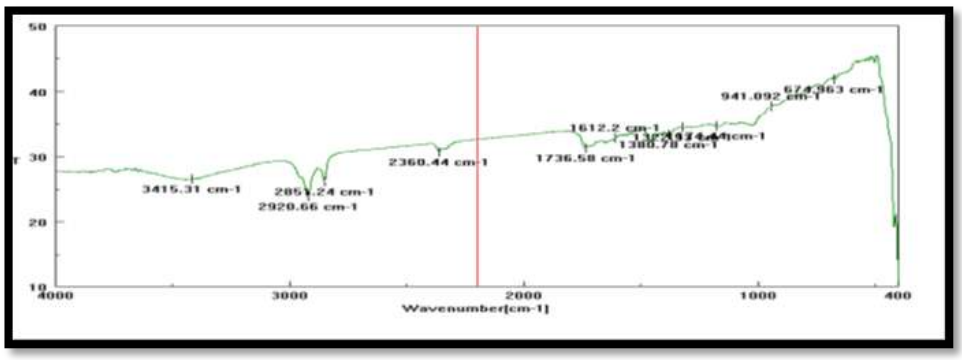
An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter.

Formula -

% Increase in pH = pH of test - pH of control/ pH of control

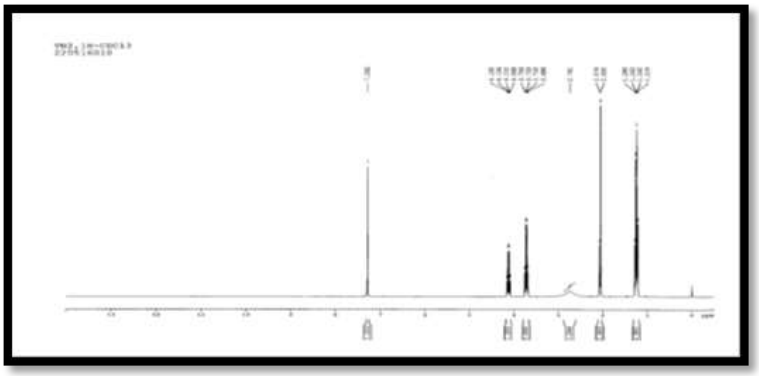
III. Result and Discussion

IR spectra obtained from A.B.C.P.Sangli



IR spectra of obtained compound

NMR was performed at Pune university, Pune



NMR of obtained compound

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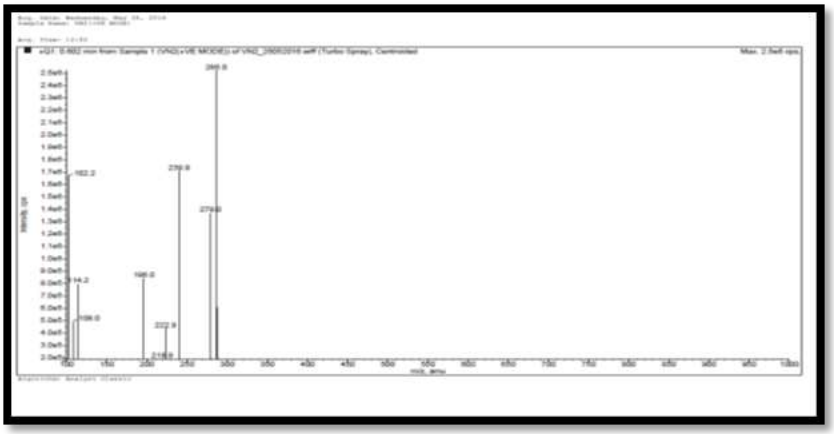


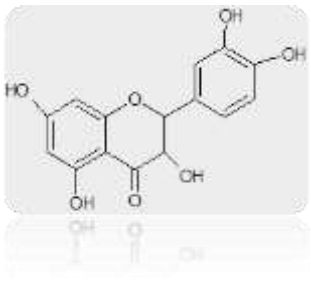
Mass spectra of obtained compound
Mass spectra was obtained at NCL, Pune.

Identification of compound- The IR of obtained compound showed absorption band at 3415.31 was O-H Streching, The absorption band at 1736.50 was showed C=O Streching, The absorption band at 1612.2 was showed C=C Streching, The absorption band at 674.96 was showed Aromatic C-H Bending. All these absorption band showed presence of myricetin which flavanoid in nature.

NMR of obtained compound showed chemical shift at δ 7.27 was represent Aromatic ring, δ 2.74 was represent hydroxyl group.

Mass spectra of obtained compound showed Molecular weight 306 and Molecular ion peak 286

From these spectral analysis we were concluded that the resulting compound may be Myricetin which was flavanoid in nature.



Effect of methanolic extracts of *Tagetes erecta* L. leaves on Ulcer index, pH, Free acidity, Total acidity

Groups	Ulcer index	pH	Free acidity	Total acidity
Normal	-	4 .60± 0.019	9.74± 0.35	20.34± 0.88
Control	4.55 0.52	1.87±0.028	40.39± 2.58	110.2 ±9.95
Higher dose	2.12±0.35	3.44±0.051*	21.63±5.00	60.19±3.45
Lower dose	2.56±0.25	3.89±0.02	25.64±4.83	68.44±2.56
Ranitidine	2.11±0.06	4.41±0.048	12.81±0.79	25.56±0.35

IV. Conclusion

The thin layer chromatography and column chromatography was used for separation of chemical constituent. From TLC and column chromatography and spectral analysis we concluded that the resulting compound may be myricetin which was flavanoid in nature.

The Pharmacological evaluation of methanolic extract of Tagetes erecta leaves showed better antiulcer activity by ethanol induced gastric ulcer.

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