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AntibacteriaL Activity and Phytochemical Study of Ethanolic Extract of *Triumfetta rhomboidea* Jacq

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Abstract: Ethanolic extract of *Triumfetta rhomboidea* Jacq was subjected to various phytochemical tests. Preparative Thin layer Chromatography study of the extract was performed and active constituents were isolated. Spectral analysis of the isolated constituent indicates that *Triumfetta rhomboidea (*Tiliaceae) contains carbohydrate glycosides, phytosterol, steroids, flavonoids, tannin & phenolic compounds and triterpenoids. Antibacterial activity of ether and alcoholic extract of the plant was performed. Results exhibited that *Triumfetta rhomboidea* Jacq contain good antibacterial action.

KEY WORDS: *Triumfetta rhomboidea* Jacq, Preparative Thin layer Chromatography, Phytochemical.

Introduction

Triumfetta rhomboidea is a perennial herb having important role in ancient therapy. Various Parts of the plant used therapeutically are fruit, flower, leaves, bark and root. Root is tonic styptic, galactogogue, aphrodisiac, cooling, useful in dysentry and as diuretic. Pounded roots are given in the treatment of Intestinal ulcer. Leaves, Flowers and Fruit are mucilaginous demulcent, astringent, and also used in gonorrhoea and against leprosy.[1-4] In the present study, active constituents of the plant were analyzed and evaluated for antibacterial activity.

Experimental

Phytochemical Screening

Triumfetta rhomboidea jacq (tiliaceae) was procured from botanical garden of B K Mody Govt Pharmacy College, Rajkot. The leaves of *Triumfetta rhomboidea* were dried under shade and powdered with a mechanical grinder. Dried material was extracted with ethanol (90% v/v) in Soxhlet apparatus and after complete extraction (50 hr) the solvent was removed by distillation under reduced pressure and resulting semisolid mass was vacuum dried.[5-14]

Ethanolic extract (EETR) of *Triumfetta rhomboidea* were subjected to preliminary phytochemical screening for the detection of various plants constituent.

Test for alkaloids

The small portion extracts were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate) Dragendorffs reagent (orange brown precipitate)

Test for carbohydrates and glycosides

Small quantity of ethanolic extract was dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to defect the absence of carbohydrates.

Another small portion of extract was hydrolyzed with dilute hydrochloric acid for few hours in water-bath and was subjected to Liebermann- Burchard's, legal and Borntrager's test to defect absence of different glycosides. (Pink to red color indicates presence of glycosides)

Test for flavonoids

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow coloration absorbed in extract indicated presence of flavonoids.

Test for steroids

2ml acetic anhydride was added to 0.5 g ethanolic extract with 2ml H_2SO_4. The color changed from violet to blue or green in samples indicated presence of steroid.

Test for terpenoids (salkowski test)
Five ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml), was carefully added to form a layer. A reddish brown coloration of the interface was formed indicated presence of terpenoids.
Test for saponin
About 1 ml of alcoholic and agrees extract was diluted with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated presence of saponin.
Test for tannin
When a drug is treated with vanillin-hydrochloric acid reagent, pink or red color is formed due to formation of phloroglucinol.
Test for protein
Mellon's reaction: Million's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating.
Test for volatile oil or essential oil
Place a thick section of drug on glass slide. Add a drop of Sudan red 3rd reagent and after two minute wash with 50% alcohol mount in glycerin.
Preparative Thin Layer Chromatography
Ordinarily, microgram quantities of mixture of organic compounds are separated by analytical TLC. It is possible to scale up the quantities to milligram amount (10-50mg) by using thicker layer (0.5 – 2.0mm thickness) of the support material and by the use of larger plates (20 x 20 cm or 20 x 40 cm). Multiple developments also bring about better resolution. Preparative TLC for the isolation of marker compound from the ethanolic extract of *Triumfetta rhomboidea* leaves was performed by using solvent system Toluene: Ethyl acetate (9:1).[15-20]
Antibacterial Activity
In the present research work, the antibacterial activity spectrum of ethanolic extract and ether extract of *Triumfetta rhomboidea* Jacq was analyzed.[21-28] (Table 3 & 4) Three Gram positive bacteria, *Staphylococcus aureus* (MTCC 737)**,** *Enterococcus faecalis* (MTCC 439)*, Bacillus cereus* (MTCC 430) and three Gram negative bacteria *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 2642), *Escherichia coli* (MTCC 1687) were used. Inoculum size was adjusted to 1 to 2 × 10^7 CFU (Colony Forming Units)/ml by serial dilution with sterilized nutrient broth media. Nutrient agar (pH 7.2-7.4) was used for routine susceptibility testing of nonfastidious bacteria. Stock solution of 10000µg/ml was prepared in 20 % v/v water in DMSO. Using the stock solution, 6000µg/ml, 4000µg/ml, 2000µg/ml and 1500µg/ml solutions were prepared from which 100 µl solution was taken for assay. Ciprofloxacin was used as a standard. 20 % v/v WFI in DMSO was used as a control. Antibacterial assay was carried out by agar Well Diffusion Method. [17-19] After 16 to 18 hours of incubation, each plate is examined.

Result and Discussion

Phytochemical screening suggests that ethanolic extract contain various constituents which are given in the table 1. Preparative TLC study revealed presence compounds COMP-01, COMP -02, COMP -03, COMP -04 and COMP -5. The compound COMP -01 to COMP -05 gives positive Knollar's and Libermann – Burchred test and the colour produced was typical of triterpences. IR spectrum produced was similar to triterpences. IR spectrum in the fundamental region also supported triterpense structure as the bands were noticed due to O-H stretching and C-H stretching of alkanes. (Table 2)

The results of preliminary evaluation showed that *Triumfetta rhomboidea* Jacq posses good antibacterial activity. *P. aeruginosa* and *E. coli* are resistant or less susceptible to *Triumfetta rhomboidea* Jacq.

Table – 1. Data showing the preliminary phytochemical screening of the two extracts of *Triumfetta rhomboidea.*

Phytochemical	Presence/Absence
Carbohydrate	++
Glycosides	++
Alkaloids	--
Phytosterol and steroids	++
Flavonoids	++
Protein& Amino Acid.	--
Tannin & phenolic compounds	++
Triterpenoids	++

Table-2. IR and UV Spectral data of the isolated samples

Code	IR data cm^{-1}	UV
01	3591 (C-H Stretching in alkenes), 2956 (C-H Stretching in the alkanes.), 1731,1701,1683 (C=O Stretching), 1286, 1334 (C-H bending vibration in the alkynes.), 898, 794, 723 (aromatic hydrocarbons.), 1014 (Diphenyl methanol.) 1201 (O-H stretching in phenol)	245
02	3670 (C-H Stretching in alkenes), 2956 (C-H Stretching in the alkanes), 1731,1716,1683 (C=O Stretching), 1222, 1271, 1340 90 (C-H bending vibration in the alkynes), 794, 729, 682 (aromatic hydrocarbons), 1222 (O-H stretching in phenol)	270
03	3151 (C-H Stretching vibration in alkenes.), 2956 (C-H Stretching vibration in the alkanes.), 1745,1735,1683 (C=O Stretching), 1253, 1286 (C-H bending in the alkynes.) 796, 757, 723, 688 (aromatic hydrocarbons) 1253 (O-H stretching)	205
04	3006,3076 (C-H Stretching in aromatic ring), 2956 (C-H Stretching in alkene), 1735,1716,1685 (C=O Stretching), 1224, 1271, 1311 (C-H bending vibration in alkyne), 793, 725 (aromatic hydrocarbons), 1224cm^{-1}(O-H stretching {phenolic})	225
05	3672,3735 (C-H Stretching), 2956 (C-H Stretching), 1733,1718,1701(C=O Stretching), 1274, 1311, 1355 (C-H bending vibration), 881, 794, 777, 723, 682 (aromatic hydrocarbons), 1213 (O-H stretching)	295

Table 3. Zone of inhibition of different concentration of ethanolic extract of *Triumfetta rhomboidea* Jacq against test microorganism.

	S. aureus	***B. cereus***	***Ent. faecalis***	***E. coli***	***Ps. aeruginosa***	***Kl. pneumoniae***
STD	39.10 ±0.95	36.67 ± 0.61	30.67± 0.61	35.60 ± 0.53	41.07± 1.01	36.53 ± 0.61
150 µg/ well	11.13± 0.76	11.20± 0.20	8.47 ± 0.42	1.00 ± 0.20	0.00	6.20 ± 0.20
200 µg/ well	22.37±0.78	25.20 ± 1.06	21.87 ± 1.20	1.70 ± 0.10	2.47 ± 0.12	21.40 ± 1.25
400 µg/ well	25.33±0.70	27.27 ± 1.10	27.07± 0.92	2.40 ± 0.20	2.80 ± 0.20	23.27 ±1.10
600 µg/ well	28.30 ± 0.95	31.47 ± 1.62	29.73±1.62	3.07 ± 0.12	3.00 ± 0.20	28.87 ± 1.03

Table 4. Zone of inhibition of different concentration of ether extract of *Triumfetta rhomboidea* Jacq against test microorganism.

	S. aureus	***B. cereus***	***Ent. faecalis***	***E. coli***	***Ps. aeruginosa***	***Kl. pneumoniae***
STD	39.10 ±0.95	36.67 ± 0.61	30.67± 0.61	35.60 ± 0.53	41.07± 1.01	36.53 ± 0.61
150 µg/ well	5.60 ± 0.72	4.33 ± 0.30	8.60±0.53	0.00	0	5.33 ± 0.42
200 µg/ well	9.30 ± 0.75	8.73 ± 0.64	11.67±0.42	0.00	0	8.33 ± 0.31
400 µg/ well	10.13 ± 0.70	10.67 ± 0.61	13.07 ± 0.61	0.00	0	8.47 ± 0.31
600 µg/ well	10.77 ± 0.95	11.67 ± 0.42	14.60±0.60	2.07 ± 0.31	3.20 ± 0.20	10.47 ± 0.42

Figure 1. Graphical presentation of Inhibition Zone of different concentration of ethanolic extract of *Triumfetta rhomboidea* Jacq against test microorganism

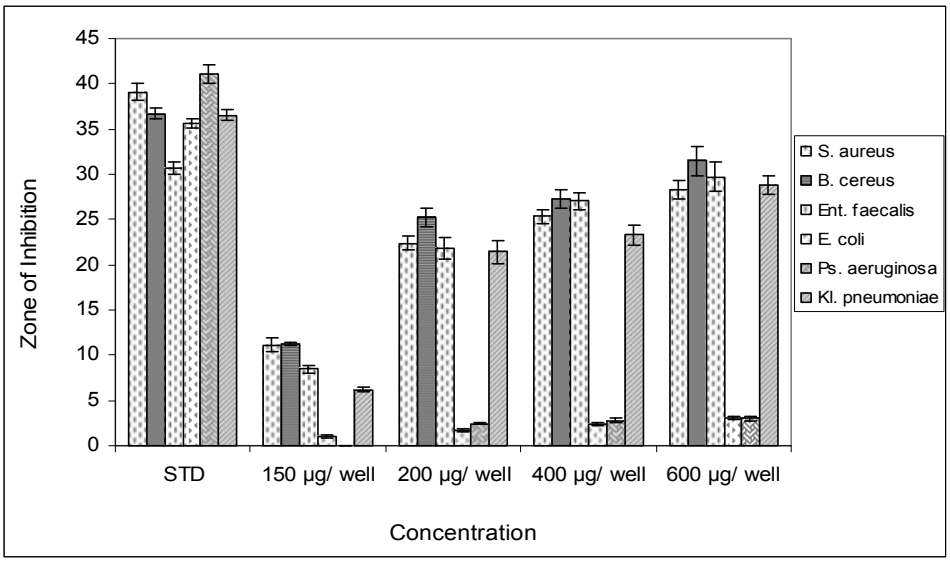
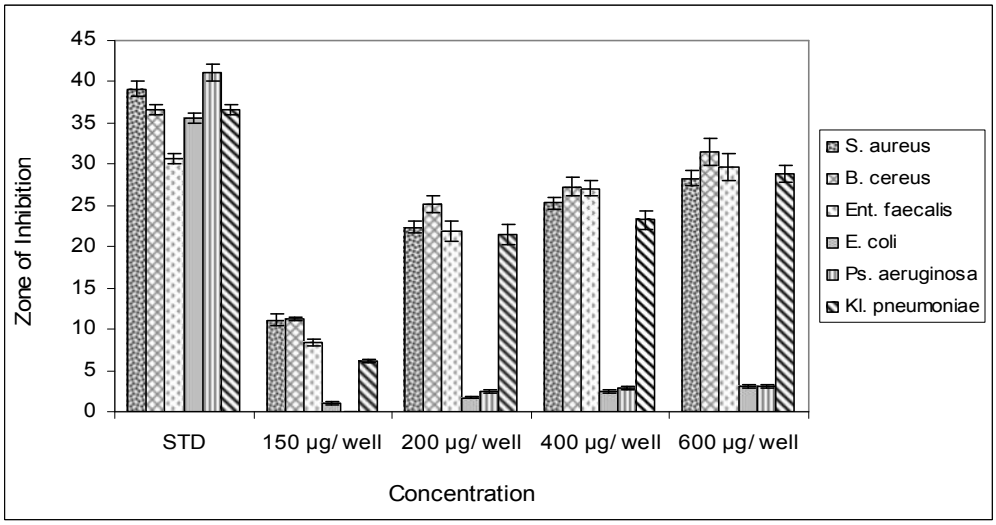


Figure 2. Graphical presentation of Inhibition Zone of different concentration of ether extract of *Triumfetta rhomboidea* Jacq against test microorganism



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