Phytochemical and Pharmacological Screening of Glycosmis pentaphylla (Retz) A.DC. (Fam. Rutaceae)

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Abstract— Glycosmispentaphylla(Retz.) A. DC. a beautifully shaped evergreen shrub from the family Rutaceae was investigated for its phytochemical & biological activities. Preliminary chemical group identification revealed the presence of alkaloids, gums, reducing sugars, tannins, flavonoids and saponins. The study was aimed to investigate the biological interest of these compounds of Glycosmispentaphylla(Retz.) A. DC. for antimicrobial & minimum inhibitory concentration, membrane stabilization, anxiolytic activity.In-vitro antimicrobial sensitivity was evaluated against 4 Gram positive and 6 Gram negative pathogenic bacteria and 7 fungi using azithromycin and fluconazole respectively as standard. In disc diffusion antimicrobial assay Glycosmispentaphylla(Retz.) A. DC. showed varying degrees of antimicrobial activities with zone of inhibition ranging from 9.26 mm to 14 mm and 10 mm to 11.33 mm for bacteria and fungi respectively where the growth of S. paratyphy and A. niger were strongly inhibited.In the minimum inhibitory concentration (MIC) test by serial dilusion method a mild to strong MIC's were observed for the test bacteria. Test extract 1mg/ml and 0.5mg/ml of Glycosmispentaphylla(Retz.) A. DC. produced inhibition of the heat induced haemolysis of RBCsby 55.00 % & 42.27% respectively whereas, the standard, aspirin showed 71.36 %. Which shows membrane stabilization activityGlycosmispentaphylla(Retz) A. DC.showed moderate anxiolytic activity in mice at the doses of 200 & 400mg/kg-body weight as compared to the standard anxiolytic agent diazepam. Sample provide % of open field cross inhibition respectively 26.49 % & 46.75 % compared to diazepam 84.24% and % of hole cross inhibition 42.86 % & 57.14 % compared to diazepam 87.01 %.

Index Terms— Glycosmis pentaphylla, Phytochemical Investigation, Antibacterial Activity, MIC, Membrane Stabilization, Anxiolytic Activity.

1 INTRODUCTION

Glycosmis pentaphylla (Retz) A. DC. is called as Jamaica mandarin orange, Orange berry (English), Ashvashakota, Vananimbuka, Pathalagarudi (Sanskrit), Ban-nimbu (Hindi), Kirmira (Marathi), Golugu and Gongipadu).Its Family: Rutaceae – Rue family and Genus: Glycosmis correa – glycosmis. This plant has been used as a medicinal plant for many years for its anti microbial, membrane stabilizing and antianxiolytic activity. This job has been done to elucidate the phytochemicals and its pharmacological activities.

1.2 Objectives

Medicinal plants possess various medicinal properties; have been serving as the major sources of therapeutic agents for maintenance of human health. These medicinal plants were used by the early human beings, as are done now, in a variety of forms, such as in the entire form, and as powders, pastes, juices, infusions and decoctions for the treatment of their various diseases and ailments.

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These various converted forms of the medicinal plants may therefore conveniently and genuinely called medicinal preparations or medicaments. This way, the medicinal plants formed an integral part of the health management practices and constituted important items of medicines used in the treatment of diseases from the very early days of human civilization.

In order to achieve these aims, the following research objectives have been identified:

a. To test the extract for the detection of different chemical groups present in the extract.
b. To evaluate the extract for this antimicrobial activity and minimum inhibitory concentrations against the test organisms.
c. To evaluate the extract for this anxiolytic activity on mice.
d. To evaluate the extract for their in-vitro membrane stabilizing activity.

The overall purpose and objective of the study is to analyze phytochemical substances present in the plant and evaluate the biological activities of ethanol extracts of Glycosmis pentaphylla (Retz.) A. DC.

2. EXTRACTION

200gm powder of the plant was taken in clean, flat-bottomed amber colored glass container and soaked in 700ml of 95% ethanol. The container with this contents was sealed and kept for several days accompanying occasional shaking. The whole mixtures then underwent coarse filtration by pieces of cotton.

Then they were filtered through filter (Whatmann) paper and the solvent was made to evaporate under the room temperature.

2.1 Yield determination

After evaporation of the solvent, the yield value of the extracts was calculated with respect to the initial amount of the powder subjected for extraction. And the yield value was 9.132% for Glycosmis pentaphylla (Retz.) A. DC.

Yield = Extract weight (gm)/ Initial powdered material (gm) × 100

=18.264 (gm)/ 200 (gm) × 100

= 9.132%.

3. Phytochemical Investigation

Plant synthesizes a wide variety of chemical compounds which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. In this study Mayer's test, Dragendorff's test, Wagner's test Hager's test, Tannic acid test were donr for determining the presence of Alkaloids, Salkowski's test, Libermann-burchard's test for Glycosides, Salkowski's test Libermann-burchard's test for steroids, Ferric chloride test, Potassium dichromate test, Keller-Killiani test for tannins, Conc. HCl and alcoholic test for falvonoids, Shake test (aq. solution) for saponins, Fehling's test, Benedict's test for reducing sugar and Molisch's test for gums.

3.1 Result of the phytochemical screening

From the above screening and tabular presentation, it should be mentioned that the ethanol extract of Glycosmis pentaphylla (Retz.) A. DC. contain reducing sugar, glycosides, tannins, alkaloids, flavonoids, saponins and gums.

4. Antibacterial Activity

4.1 Principle:The assigned assay method is based on the ability of antibiotics to diffuse from a confined source through the gel media and create a concentration gradient. If the agar seeded or streaked with a sensitive organism, a zone of inhibition will result. In this method, a definite amount of the test sample was dissolved in definite amount of solvent to give solution of given concentration (µg/µl). Then the sterile filter paper discs having 5mm in diameter were impregnated aseptically with known amount of the test substances and dried. Such discs and standard antibiotic discs were placed on plate containing a suitable medium seeded with the test organisms. These plates were kept at low temperature (4°c) for 24hrs to allow maximum diffusion. The dried discs absorb water from the agar medium and the material under test was dissolved. The plates were then kept in an incubator (37°C) for 18hrs [1], [2], [3] to allow the growth of organisms. If the test material has antibacterial activity, it will inhibit the growth of microorganism having a clear distinct zone called "Zone of Inhibition". The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in term of mm.

4.2 Preparation of test samples

The ethanol extract of Glycosmis pentaphylla (Retz.) A. DC. was subjected for antibacterial evaluation. In order to free from microbial contamination the cold extract was filtered through sterilized filter (Whatmann) paper, and the hot extract was subjected for direct sterilization by autoclaving.The test samples were prepared by dissolving the extracts of the samples in ethanol. The concentration was 50µg/µl. In agar plate disk diffusion method dilutions having 10µl of ethanol extract was placed on the discs and for complete evaporation of the solvent, the discs were incubated as mentioned above. This concentration also used for making standard disc i.e. azithromycin 2.5µg/µl.

4.3 Placement of discs and incubation

The discs (both sample & standard) were subsequently impregnated centrally into the agar gel separately with the help of a sterile forceps to assure complete contact with the previously cultured medium surface. The plates were then inverted and kept in a refrigerator for about 24hrs at 4°c. This is sufficient time for the material to diffuse to a considerable area of the medium. Finally, the plates were incubated at 37°c for 18hrs.The test was performed three times and the mal-growth as well as uneven zone of inhibition was detected and repeated for accuracy.

Table - 1 : In-vitro antibacterial test of crude extract, standard and control.

Table - 2 : Zone of inhibition by treatment groups

Tested Bacteria	Diameter of zone of inhibition [mm]								
	EEGP (50µg/µl)				Standard (Azithromycin) (2.5µg/µl)				Control Ethanol (10µl)
	T1	T2	T3	Mean	T1	T2	T3	Mean	
Gram positive species									
S. aureus	11.67	11.35	11. 5	11.89	18	16	20	18	-
B. cereus	0	0	0	0	12	12	13	12.33	-
B. subtilis	0	0	0	0	16	14	15	15	-
B.megaterium	9.33	9.25	9.19	9.16	21	21	20	20.67	-
Gram negative species									
V. cholera	9	8.67	8.95	8	19	21	21	20.33	-
S. typhi	10	10.5	10.35	10.28	15	16	16	15.67	-
S. paratyphi	11	11.45	11.35	11.27	21	20	21	20.67	-
S. dysenteriae	12	13.33	10.33	11.89	16	16	14	15.33	-
E. coli	0	0	0	0	17	16	17	16.67	-
P. aeruginosa	10	11	10.5	10.5	15	18	18	17	-

Microorganisms	Zone of inhibition (MZI±SD) mm	
	EEGP	Reference standards
Gram positive species		
B.aureus	11.39□□□□	14.8±1.04
B.cereus	0	12.3±0.58
B.subtilis	0	12.8±1.26
B. megaterium	9.26□□□□□	14.2±0.76
Gram negative species		
P. aeruginosa	10.5□0.35	11.3±1.04
E. coli	0	15.5±0.50
S. dysenteriae	11.89□1.06	14.7±0.58
S. typhi	12±1[d]	12.5±0.5
V. cholerae	13±1[c]	13.8±0.29
S. paratyphi	14±1[c]	12.5±1.50

[a]p<0.01, [b]p<0.05 [c]p<0.10, [d]p<0.50 ;SD: Standard deviation;EEGP: Ethanol extract of *Glycosmis pentaphylla*; MZI: Mean zone of inhibition (mm); zone of inhibition under 8 mm were considered as less active and were discarded.

4.4 Determination of the antibacterial activity

The antibacterial sensitivity of the crude ethanol extract was determined by statistical evaluation (ap<0.01, bp<0.05 cp<0.10, dp<0.50) of obtained zone of inhibition (mm) and comparing

the result with the standard, azithromycin which actively inhibited the growth of all the test bacterial strains at a concentration of 2.5µg/µ1.

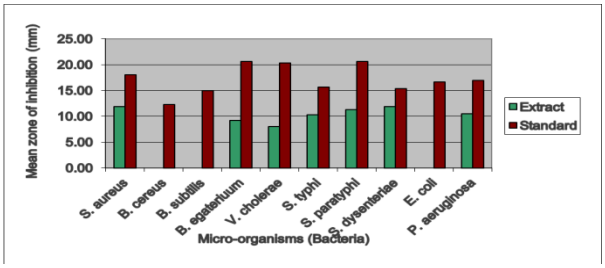

Fig. 1: Zone of inhibition of test bacteria by treatment groups

4.5 Result of antibacterial screening

In the antibacterial sensitivity test, it was observed that the plant extract produced significant zone of inhibition. The highest zone of inhibition was produced by Glycosmis pentaphylla (Retz) A. DC. (14mm)(dp<0.50) against Salmonella paratyphi at the dose of 50µg/µ1. The significant growth of inhibition was produced by the dose 50µg/µ1 of EEGP is 8 to14 mm, but it was inactive against Bacillus cereus, Bacillus subtilis and Escherichia coli. On the other hand the EEGP at the dose of 50µg/µ1 showed zone of inhibition 9.26 to 14±1cmm to all the pathogens except the pathogenic strains Bacillus cereus, Bacillus subtilis and Escherichia coli. Azithromycin was active against all the test bacteria within the range of 27.17 to 31.33mm.

From the table- 1 and experimental data manipulation, it was found that the plant EEGP showed maximum inhibition to the gram negative pathogen.

5. Minimum Inhibitory Concentration (MIC) Determination

Lowest dose of an antibiotic that does the inhibition of the growth of a particular no(s) organism(s) upon which it acts is called the 'Minimum Inhibitory Concentration' (MIC). The present study protocol was designed and performed due to the fact that the extract of Glycosmis pentaphylla (Retz.) A. DC. was found as positive antimicrobial sensitivity and the extract showed activity against a number of human pathogenic bacteria. For MIC test the 'Serial tube dilution technique' [4] was selected. For bacteria, readymade nutrient broth medium (NBM) was dissolved in double distilled water (DDW) to obtained required volume of medium (13gm for 1000ml). Then the mixture was dissolved in DDW and made up to required volume. The media was then transferred to the screw cap tube which was then sterilized in an autoclave at a temperature of 121°C and a pressure of 15Ibs/sq. inch for 30min.

5.1 Experimental design

1. Eleven (11) screw-cap test tubes were taken and serially marked T1, T2, T3, T4, T5, T6, T7 for sample solutions and the rest four: Tm for medium, Tmi for medium & inoculums, Tms for medium & solvent and Tst for standard.
2. 1ml of nutrient broth medium was taken in all test tubes and sterilized in an autoclave at a temperature of 121°C and a pressure of 15Ibs/sq. inch for 30minutes.
3. 1ml of 1000µg sample was added to the no-1 marked tube and the tube was shaken gently for proper mixing of the content.
4. 1ml of the content form the T1 was added to the T2 and that action was continued up to the no.T7 marked tube. In the end, after proper mixing 1ml content from the T7 was discarded.
5. 100µl of specified bacterial suspension (inoculums containing 106cfu/ml of cells) of the clinical pathogen were added to the test tube, Tmi and seven extract containing tubes by a suitable micropipette (2-20µl).
6. For Tms only 1ml ethanol was added, after shaking 1ml of the mixture was discarded from the tube. Tm contained only 1ml medium.
7. All the test tubes were subjected for incubation. In case of antibacterial activity test, the tubes were incubated for 18h at 37°C. All the tests were performed three times and the uneven growth and inhibition was checked and repeated for accuracy.

5.2 Minimum inhibitory concentrations determination

The minimum inhibitory concentrations for the plant species G. pentaphylla (Retz) A. DC. was determined by the inhibitory action on the growth and survival of the clinical organisms. Here the growth inhibition was visually observed by naked eye. The more the growth of the organisms within the test tubes the more the turbidity of the reaction mixtures.

5.3 MIC of extract of G. pentaphylla (Retz) A. DC.

Table - 3 : Growth inhibitory concentrations by ethanolic extract of Glycosmis pentaphylla (Retz) A. DC.

Table - 4: Minimum zone of inhibition(µg/ml) by crude extract

Microorganisms	Culture tubes for sample (µg/ml)							T_{mi}	T_m	T_{ms}	T_{st}
	T_1	T_2	T_3	T_4	T_5	T_6	T_7				
B. megaterium	+	-	-	+	+	+	+	+	-	-	-
S. aureus	+	-	-	+	+	+	+	+	-	-	-
S. dysenteriae	+	+	-	+	+	+	+	+	-	-	-
S. paratyphi	+	-	+	-	+	+	+	+	-	-	-
S. typhi	+	+	+	-	+	+	+	+	-	-	-
V. cholerae	+	-	-	+	+	+	+	+	-	-	-
P. aeruginosa	+	-	-	-	+	+	+	+	-	-	-

T_1 = 500µg/ml, T_2 = 250µg/ml, T_3 = 125µg/ml, T_4 = 62.5µg/ml, T_5 = 31.25µg/ml, T_6 = 15.625µg/ml, T_7 = 7.8125µg/ml, Tmi = tubes for media + inoculums; Tm = tubes for media; Tms = tubes for media + solvent and Tst = tube for standard.

Microorganisms (Bacteria)	Minimum zone of inhibition(µg/ml) EEGP
B. megaterium	125
S. aureus	62.5
S. dysenteriae	125
S. paratyphi	62.5
S. typhi	62.5
V. cholera	125
P. aeruginosa	62.5
EEGP: Ethanol extract of Glycosmis pentaphylla (Retz.) A. DC	

5.4 Result of the in-vitro minimum inhibitory concentration test

The ethanolic extract of Glycosmis pentaphylla (Retz.) A. DC. showed minimum inhibitory concentration in case of bacteria:

B. megateriuum, S. dysenteriae, V. cholera at the concentrarion of 125(µg/ml) and S. aureus, S. paratyphi, S. typhi and P. aeruginosa at the concentrarion of 62.5 (µg/ml).

5.5 Results of antimicrobial activity

From the overall view of the experimental data, it can be concluded that G. pentaphylla (Retz.) A. DC. possess a significant antibacterial activity and antifungal activity. The antimicrobial activities of the extract was evaluated by the disc diffusion method against 4 Gram positive and 6 Gram negative pathogenic bacteria using azithromycin as standard. In the screening, the extract showed significant antimicrobial activity against S. paratyphi , V. cholera, S. typhi, Shigella dysenteriae, P. aeruginosa and S. aureus with zone of inhibition 14±1mm, 13±1mm, 12±1mm, 11.89±1.06mm, 10.5±0.35mm and 11.39±0.1mm respectively compared to the reference standard Azithromycin (12.5±1.50mm, 13.8±0.29mm, 12.5±0.50mm, 14.7±0.58mm, 11.3±1.04mm and 14.8±1.04mm respectively) and moderate activity against B. megaterium with zone of inhibition of 9.26±0.04 mm compared to the reference standard azithromycin (14.2±0.76) (Table – 6c).

In case of antifungal activity, the ethanol extract showed good inhibition activity at the dose of 50µg/µl with zone of inhibition of 11.33±0.4083mm, 10±0.7072 mm, 10±1.2249 mm and 11±0.7072 mm against the A. niger, B. dermatitidis, C. albicans and C. neoformans. (Table – 6f).

The ethanol extract of Glycosmis pentaphylla (Retz.) A. DC. showed minimum inhibition of bacteria at the concentration of 62.5 and 125 µg/ml against S. paratyphi, S. typhi, P. aeruginosa , S. aureus and B. megateriuum, S. dysenteriae, V. cholera respectively.(Table – 6h)

6. Assessment of In-vitro Membrane Stabilization Activity

The ethanol extract of Glycosmis pentaphylla (Retz) A. DC. was made to test for the in-vitro membrane stabilization in the authentic model for the face of the new and newer agents that counts the desired activity.

The present study was developed by certain modification of the method claimed by Mizushima Y and Kobayashi M., 1968 on the basis of in-vitro determination of the membrane instability caused by the control group and comparing it with the positive control and test groups. The more the membrane instabilization effects, the more it will cause haemolysis and the measured absorbance will be higher.

For standard (0.1mg in 1ml), 10mg acetyl salicylic acid was dissolved in 100ml double distilled water (DDW). To prepare iso and hypo-saline, 900mg and 500mg NaCl were added to 100ml of DDW respectively. For 10% RBCs suspension, fresh whole human 1ml blood was collected and diluted with RBC diluting fluid up to 20ml. then 2ml diluted was transferred to the 18ml normal saline solution to get the 10% RBC suspension. [5].For test groups, 65mg of crude extract was mixed with measured volume of ethanol.

Fresh whole human sodium citrated blood was collected and used readily to perform the work.

6.1 Experimental design

For this study, 18 clean centrifuge tubes were taken. Three for standard, three for control and six for the two crude ethanol extracts. The tubes were marked accordingly. 1ml of 10% RBCs suspension were kept into all treatment tubes, 1ml ethanol and 1ml acetyl salicylic acid were added to the control and positive control tubes respectively. On the other hand for test groups 1ml of (65mg/kg) ethanol extracts were mixed to the test groups as marked. Then all the tubes were treated with 1ml of hypotonic solution. The pH (7.4±0.2) of the reaction mixtures was adjusted by phosphate buffer.

All the centrifuge tubes containing reaction mixture were incubated in a water bath at 37°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 3000rpm for 10min. After cooling and filtering (Whatmann filter paper) the absorbance of the supernatants were taken at 556nm. The test was repeated for three times [6].

Table - 5: Spectrophotometric determination of membrane stabilization

Test groups	Absorbance at 546nm	Mean absorbance	% MHLs	%MIHLs	t-test (p-value)
Control (DW)	0.61	0.61	100	0	-
	0.6				
	0.62				
Standard ASA Conc. 0.1mg/ml	0.45	0.45	28.64	71.36	18.90 (<0.00001)
	0.47				
	0.43				
EEGP Conc. 1mg/ml	0.52	0.52	45.00	29.09	6.38 (<0.00001)
	0.48				
	0.56				
EEGP Conc. 0.5mg/ml	0.59	0.58	57.73	20.45	8.40 (<0.0001)
	0.58				
	0.58				

*ASA = Acetyl Salicylic Acid, MHLs = Mean haemolysis, MIHLs = Mean inhibition of haemolysis, EEGP: ethanol extract of *Glycosmis pentaphylla* (Retz) A. DC.

Table - 6: Tabulation for in-vitro membrane stabilization test

Test groups	SD	SEM	Total inhibition of haemolysis
Control	0.025	0.018	00.00±0.018
Standard, Conc. 0.1mg/ml	0.03	0.021	71.36±0.021[a]
EEGP, Con. 1mg/ml	0.04	0.028	55.00±0.028[a]
EEGP, Conc. 0.5g/ml	0.006	0.0041	42.27±0.0041[b]

*SD = Standard deviation, SEM = Standard error of mean, Inhibition of haemolysis = %IMHLs±SEM, [a]p<0.00001, [b]p<0.0001, EEGP: ethanol extract of *Glycosmis pentaphylla* (Retz) A. DC.

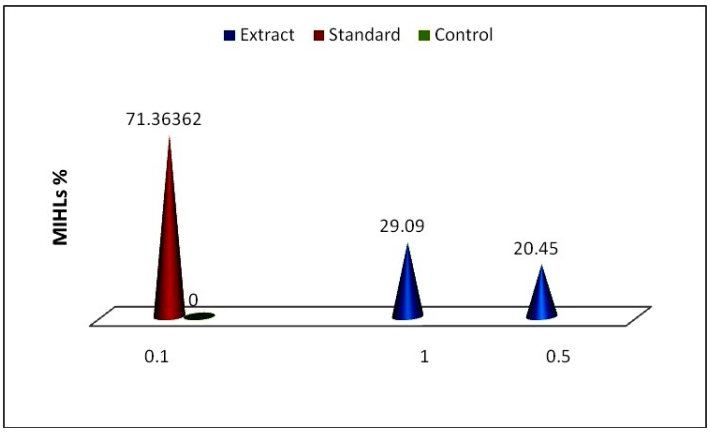

Fig. 2: Inhibition of hemolysis by treatment groups

6.2 Determination of in-vitro membrane stabilization activity

Membrane stabilization activity was measured by measuring the absorbance of the treatment group and converting it into total inhibition of haemolysis.

% Inhibition of haemolysis= (Abscontrol – Abssample) / Abscontrol × 100

6.3 Result of membrane stabilization activity

Test extract 1mg/ml and 0.5mg/ml of Glycosmis pentaphylla (Retz.) A. DC. inhibited the heat induced total haemolysis of RBCs by 55.00±0.028 & 42.27±0.0041 respectively whereas, the standard, aspirin showed 71.36±0.021 (table - 8b). The stabilization activity for crude ethanolic extract of Glycosmis pentaphylla (Retz.) A. DC. was produced a moderate membrane stabilization activity as summarized in the tabular presentation (table-8a). Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is thought that the ethanol extract of the plant may p inhibit the release of lysosomal content of neutrophils at the site of inflammation [7]. That provides evidence for moderate membrane stabilization as an additional mechanism of their anti-inflammatory effect.

7. Study of Anxiolytic Activity by Benzodiazepines

Swiss-albino mice (ICDDR, B) of either sex, average weight 19-25gm were employed in the experiment taking three in a group. For control, positive control and test group the suspending agent, Diazepam & sample (1mg/kg) was introduced by I.P. The mice were kept separately in wooden cages. After 30 min observation was collected for 5 min. Healthy adult Swiss albino mice of either sex weighing about 20-25 grams each were used for the study. The animals were divided into 4 groups of 3 animals each. The animals were housed in colony cages and maintained under standard environmental conditions, 25±2 centigrade temperature , RH 45-55 % and 12:12 h light: dark cycle . They were provided free access to food and water ad libitum. Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group III and group-IV consisting of 3 mice in each group. Each group received a particular treatment i.e. control, positive control and the two dose of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly

7.1 Preparation of samples for test, standard and control groups

For test group, the ethanol extract of Glycosmis pentaphylla (Retz) A. DC. at the dose of 200 mg/kg and 400 mg/kg were selected.

For standard (1.0 mg/kg), 0.02 gm of daizepam was taken and a suspension of 10 ml was made.

For control group, distilled water was given.

7.2 Methodology

7.2.1 Grouping

A total of 12 animals were divided into 4 groups (I, II, III, and IV).

Group I: normal control group- was received distilled water.

Group II: positive control group- was received diazepam 1mg/kg i.p.

Group III: was received ethanol extracts of Glycosmis pentaphylla leaves at dose of 200 mg/kg (oral).

Group IV: was received ethanol extracts of Glycosmis pentaphylla leaves at dose of 400 mg/kg (oral).

7.2.2 Hole Cross Test

The method was carried out as described by Takagi et al. A partition was fixed in the middle of a cage of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The mice were divided into control, positive control, and test groups containing 3 mice each. The test groups received ethanol extract of Glycosmis pentaphylla at doses of 100 and 200 mg/kg body weight orally whereas the control group received vehicle (water) and positive control received diazepam (1.0 mg/kg-1 i.p.). The number of passages of a mouse through the hole from one chamber to the other was counted for a period of 5 min at 30 min after oral administration of both doses of the test drug. [8]

7.2.3 Open field test

The open field test is one of the tests used to observe general motor activity, exploratory behaviour and measures of anxiety. The open field area was made of plain wood and consisted of a square area (45×45×20 cm). The floor had a square sheet of wood (45×45 cm) with the surface divided into sixteen small squares. Mice were divided into four groups of 3 mice and treated similarly as described in hole cross test. Thirty minutes after treatment, mice of both the control and treated groups were placed individually in the center of the open field and behavioral activities were videotaped for five minutes. Subsequently, hand operated counters and stopwatches were used to score the following behavioural parameters for a period of five minutes : (1) the number of entries and time spent in the centre, (2) periphery and corners of the field, (3) the number of crossings (number of square floor units entered) as a measure of distance traveled, (4) rearing (number of times the animal stood on hind legs) and (5) assisted rearing (forepaws touching the walls of the apparatus). [9]

7.4 Determination of the anxiolytic activity

Anxiolytic activity was determined by comparing the percent open field cross inhibition and hole cross inhibition by the extract of Glycosmis pentaphylla (Retz.) A. DC. in comparison to the control and positive control groups.

Table - 7: Experiment profile to assess the effect of crude extract on anxiolytic activity

Clinical groups	No. of mice	BW (gm)	HCC	MHC	% HC	% HCI	SEM	No. of HC	t- test (P-values)
Control (water)	1	20	24	25.67	100	0	1.47	77±1.47	-
	2	21	28						
	3	23	25						
Standard Diazepam (1mg/kg)	1	20	5	3.33	12.99	87.01	1.08	10±1.08	12.23 (<0.0005)
	2	23	3						
	3	23	2						
EEGP (200mg/kg)	1	23	13	14.67	57.14	42.86	1.08	44±1.08	6.02 (<0.002)
	2	20	16						
	3	23	15						
EEGP (400mg/kg)	1	21	9	11	42.86	57.14	1.41	33±1.41	7.18 (<0.0005)
	2	21	13						
	3	20	11						

*[BW = Body weight, HCC = hole cross count, MHC = Mean hole cross, HCI = hole cross inhibition, No. of hole cross = (MHC×3 ± SEM), SEM = Standard Error of Mean],
EEGP: *Glycosmis pentaphylla* (Retz.) A. DC.

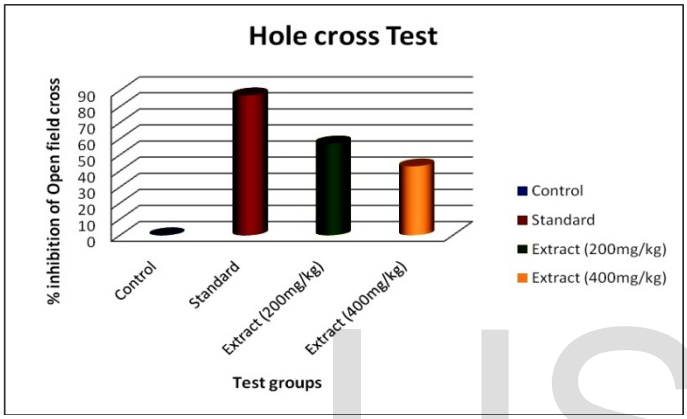

Fig. 3: % inhibition of Hole crosses by the treatment groups

Table - 8: Effect of ethanol extract of Glycosmis pentaphylla (Retz) A. DC. on the number of hole crossed in the hole cross apparatus in mice

Clinical groups	No. of mice	BW (gm)	OFCC	MOFC	%OFC	%OFCI	SEM	No. of OFC	t- test (P-values)
Control (water)	1	20	25	251.67	100	0	2.042	755±2.042	-
	2	21	25						
	3	23	25						
Standard Diazepam (1mg/kg)	1	20	37	39.67	15.76	84.24	1.780	119±1.780	78.27 (<0.0005)
	2	23	42						
	3	23	40						
EEGP (200mg/kg)	1	23	17	185	73.51	26.49	6.29	555±6.29	10.09 (<0.00001)
	2	20	19						
	3	23	18						
EEGP (400mg/kg)	1	21	127	134	53.25	46.75	4.64	402±4.64	23.22 (<0.00001)
	2	21	135						
	3	20	140						

*[BW = Body weight, OFCC = Open field cross count, MOFC = Mean open field cross, OFC = Open field cross, OFCI = Open field cross inhibition, No. of open field cross = (MOFC×3 ± SEM), SEM = Standard error of mean], EEGP: *Glycosmis pentaphylla* (Retz.) A. DC.

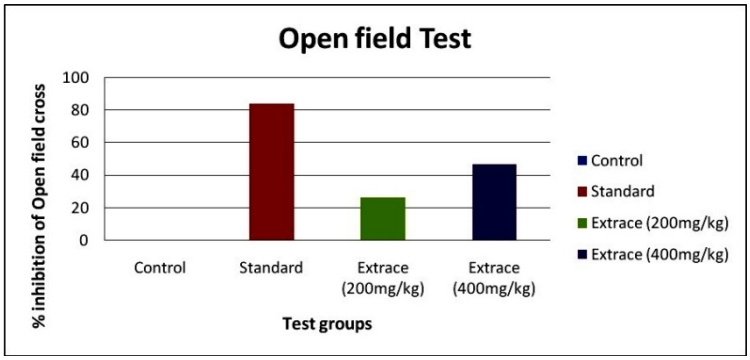

Fig. 4: % inhibition of Open field by the treatment group

7.5 Result of the anxiolytic activity

Glycosmis pentaphylla (Retz) A. DC. showed anxiolytic activity in mice at the doses of 200 & 400mg/kg-body weight as compared to the standard anxiolytic agent diazepam. Sample provide % of open field cross inhibition respectively 26.49 % & 46.75 % compared to diazepam 84.24 % and % of hole cross inhibition 42.86% & 57.14 % compared to diazepam 87.01%
The ethanol extract of Glycosmis pentaphylla (Retz) A. DC. showed moderate anxiolytic activity.

8. Conclusion

The present project work was conducted to evaluate some pharmacological properties such as antimicrobial, anti-inflammatory, membrane stabilization, anxiolytic, anti-pyretic and anti- amylase activity based on ancient practices and traditional uses of the Glycosmis pentaphylla (Retz.) A. DC.. The investigation was also done for preliminary idea about the presence of different chemical groups. The ethanol extract of Glycosmis pentaphylla (Retz.) A. DC. showed antimicrobial, membrane stabilization, anxiolytic activity. As all the experiments were conducted with some limitations, further pharmacological and toxicological study is required to establish the therapeutic uses of the plant.

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