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(RESEARCH ARTICLE)

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Phytochemical and pharmacological evaluation of anti-acne activity of herbal extract of *Cassia angustifolia*

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Abstract

P. acnes, as the foremost causative microorganism, M. furfur (yeast), *S. Epidermidis* are present in acne lesions. So in the present study ethanolic extract of *Cassia angustifolia* were selected for the anti-acne activity. Results of phytochemical screening of *Cassia angustifolia* flavonoid, proteins, alkaloids, saponins and diterpenes were detected in ethanol extracted. All phytochemical are absent in pet. ether extract of *Cassia angustifolia* . In thin layer chromatography from the Rf value (0.60) it was confirmed the presence of Quercetin as flavanoids compound in the extract. The preliminary phytochemical study was carried out according to standard literature. This revealed that they contain various phytoconstituents which can be responsible for the anti-acne activity. The acne-like inflammatory activity was carried out by measuring the ear thickness. Ethanolic leaves extract of *Cassia angustifolia* showed a significant reduction in the ear thickness. It seems that the increased ear thickness and inflammation caused due to various biochemicals, viz. various kinins, histamine and 5-HT is significantly reduced. The presence of various phytoconstituents including the hydroalcoholic leaves extract of *Cassia angustifolia* i.e. flavanoids, phenols showed significant anti-acne properties.

Keywords: *P. acnes*; *Cassia angustifolia*; Ethanolic extract; Phytochemical screening; Anti-acne properties

1. Introduction

Acne is considered as one of the most widespread skin diseases1. When extreme disfiguration occurs, it results in the development of severe consequences among the young people and may result in depression and suicide. Acne is a chronic inflammatory disease of the pilosebaceous follicle that affects about 85 % of adolescents. It is estimated that the prevalence of the disease is about 1- 12 % in the adult males and 12-17% in adult females. It is more frequent and severein males, but more persistent in women. The disease has four main causes: sebaceous hyperplasia and hyperseborrhoea; hyperkeratinization and consequent keratinocyte accession; colonization of *Propionibacterium acnes* (*P. acnes*) and respective *Staphylococcus albus* and inflammation and immune response. The production of sebum by sebocytes is stimulated by androgens, such as testosterone, which in turn stimulate the production of sebocytes. The defects in the differentiation of keratinocytes and scaling result in increasing its stickiness, are the cause of clogging of the follicle, which prevents the flow of sebum and leads to the formation of the blackhead. In 2010, it was reported that acne affects approximately 9.4% of the population. It affects about 90% of people during teenage years and sometimes in adulthood. About 20% people have moderate and severe cases. Acne rates are low in rural areas and it may not occur in the non-westernized people of Paraguay and Papua New Guinea. It is more common in females 9.8% compared to males 9.0%. Acne develops as a result of bacterial overgrowth and inflammation in the pilosebaceous units. The body's hormone level alter pilosebaceous gland functionand causes acne. Follicular epithelial cells abnormally differentiated and forms tighter intracellular adhesions and shed less. The acne spreading depends on pilosebaceous gland density and morphology and it is common in the face, chest, neck and back. Non-inflammatory acne is characterized by the

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formation of closed or open comedones. Open comedones also known as blackheads shows dark colored hyperkeratotic plugs within the follicular opening which is related to the melanin oxidation but not dirt, as it is a common disbelief. *Cassia angustifolia* (senna) and it belongs to Leguminosae family. Senna is used for the treatment of constipation mostly in Eastern and Western countries 40-41. The laxative activity of senna is due to the presence of two anthraquinone glycosides, i.e., sennoside A and sennoside B. C. angustifolia is also composed of rhein-8- diglucoside, sennosides C and D, rhein,rhein-8-glucoside,aloe-emodinand anthrone diglucoside, and napthalene glycosides such as tinnevellin glycoside and 6- hydroxy musizin glycoside, flavonoid (kaempferol), phytosterols, resin, and calcium oxalate. Senna leaves are delicate and grayish-green. The pods and fruitsareoblongin shape. The compound leaves are composed of 5-8 pairs of oval-lanceolate leaflets (2.5 cm×1.5 cm). Flowers are large and yellow.

2. Material and methods

Leaves of *Cassia angustifolia* were collected from ruler area of Bhopal, month of February, 2022. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant. After procurement of plant material, they were cleaned properly. The cleaning process involved the following steps. Very first the decayed or deteriorated plant material was removed. This was followed by washing with tap water and distilled water. The washed plant material was wrapped in blotting paper in order to remove extra water. After procurement of plant material, they were cleaned properly. The cleaning process involved the following steps. Very first the decayed or deteriorated plant material was removed. This was followed by washing with tap water and distilled water. The washed plant material was wrapped in blotting paper in order to remove extra water.

Figure 1 Dried leaves of *Cassia angustifolia*

Figure 2 Soxhlet apparatus

The dried plant part was finely powdered using electric grinder, sieved and packaged in polyethylene bags until when needed. Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs51-52. The shade dried leaves of *Cassia angustifolia* (52.6 gm) were extraction with petroleum ether using Soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. Defatted plant material was

extracted in ethanol solvent53. Powdered plant materials were extracted by Soxhlet apparatus using ethanol as solvent. The resultant content was filtered with Whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6×2 cm) and stored in a refrigerator (4°C), till used for analysis.

2.1. Phytochemical examinations were carried out extracts as per the following standard methods54.

- Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

- Detection of carbohydrates: Extracts were dissolved individually in5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

- Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Finding of pink to blood red colour indicates the presence of cardiac glycosides.

- Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20mlandthiswasshaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

- Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

- Detection of flavonoids

Lead acetate Test: Extracts were treated with few drops of lead acetate solution.

Formation of yellow colour precipitate indicate the occurrence of flavonoids.

- Detection of proteins

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

- Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicate the presence of diterpenes.

2.2. Separation and Identification of phytoconstituents by TLC

In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors

Determination of total flavonoids content was based on aluminium chloridemethod56. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

2.3. Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined

against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

2.4. Well diffusion method

The well diffusion method was used to determine the anti-acne activity of the extract prepared from the *Cassia angustifolia* usingstandardprocedure42.There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculums. The plates were incubated at 37℃ for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

2.5. *In Vivo* Anti-acne activity

Animals:- Wistar rats (150-200g) were group-housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. A separate group (n=6) of rats was used for each set of experiments.

2.6. Experimental designs

- Group –I: Control (acne induced)
- Group -II: Ethanolic leaves extract of *Cassia angustifolia* (100mg/kg, p.o.)
- Group –III: Ethanolic leaves extract of *Cassia angustifolia* (200mg/kg, p.o.)
- Group –IV: Clindamycin (200mg/kg, p.o.)

In our *in vivo* experiments for assessment of animals was divided into four groups of 6 animals each. The group I received a subcutaneous injection of 140 µg of heat- killed bacteria. Groups II, III, and IV received 100 mg/kg and 200 mg/kg of Ethanolic leaves extract of *Cassia angustifolia* and Clindamycin (200 mg/kg p.o.), respectively.

2.7. Measurement of ear thickness

Ear thickness was measured as an index of inflammatory strength and acne. Thickness was measured by using a vernier caliper. Thickness was measured once every day for the first week of induction, then every other day until the 10thday.

2.8. Statistical analysis

All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

3. Results and discussion

3.1. Results of extractive values

Table 1 Extractive values of leaves extract of *Cassia angustifolia*

S. No.	Extracts	Colour	Physical nature	% Yield* (W/W)
1.	Petroleum ether	Dark green	Solid	1.54%
2.	Ethanol	Green	Solid	6.49%

Table 2 Result of phytochemical screening of *Cassia angustifolia*

S. No.	Constituents	Pet. ether extract	Ethanol extract
1.	Alkaloids		
	Hager's Test	-Ve	+Ve
2.	**Glycosides**		
	Legal's Test	-Ve	-Ve
3.	**Flavonoids**		
	Lead acetate Test	-Ve	+Ve
	Alkaline test	-Ve	-Ve
4.	**Diterpenes Copper acetate Test**		
	Copper acetate Test	-Ve	+Ve
5.	**Phenol**		
	Ferric Chloride Test	-Ve	-Ve
6.	**Proteins**		
	Xanthoproteic Test	-Ve	+Ve
7.	**Carbohydrate**		
	Fehling's Test	-Ve	-Ve
8.	**Saponins**		
	Froth Test	-Ve	+Ve
9.	Tannins		
	Gelatin test	-Ve	-Ve

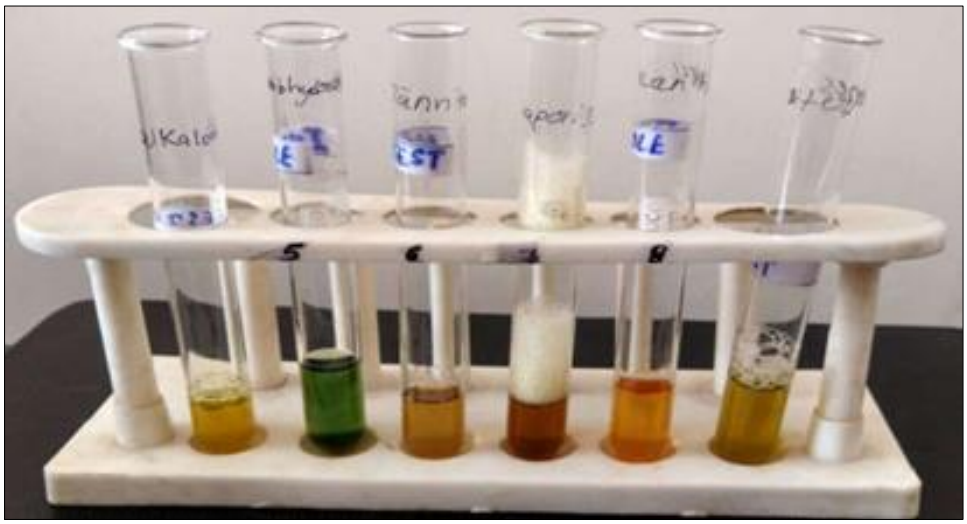

Figure 3 Phytochemical screening of *Cassia angustifolia*

3.2. Results of thin layer chromatography of leaves of *Cassia angustifolia*

Table 3 TLC of leaves of *Cassia angustifolia*

S. No.	Extract	Rf Value
	Mobile phase (Toluene: Ethyl acetate: Formic acid; 5:4:1)	
1.	Quercetin	0.60
	Leaves of *Cassia angustifolia*	
	Long UV	0.62, 0.70, 0.76
	Short UV	0.62, 0.70
	Normal light	0.62, 0.70

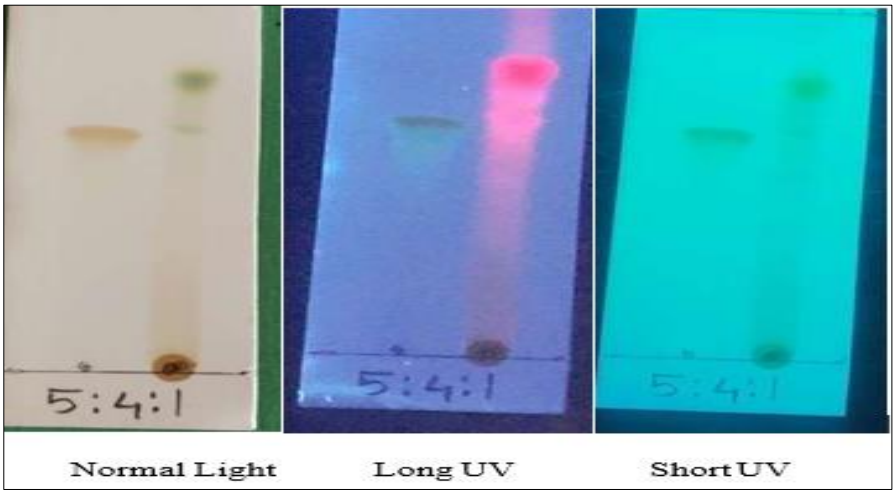

Figure 4 Thin layer chromatography (Quercetin)

3.3. Calibration curve of Quercetin

Table 4 Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.191±0.002
2	10	0.348±0.003
3	15	0.514±0.001
4	20	0.652±0.002
5	25	0.812±0.003

*Average of three determination, Mean ± SD

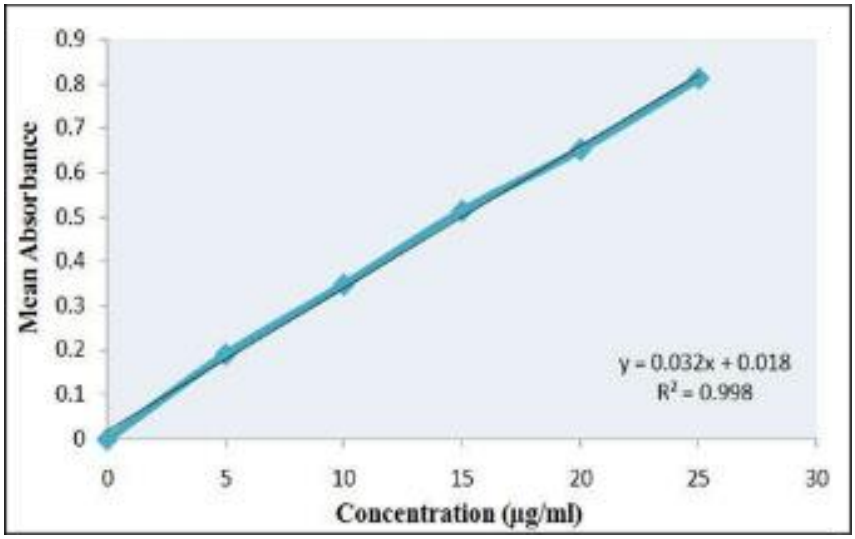

Figure 5 Graph of calibration curve of Quercetin

Table 5 Preparation of calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean Absorbance
1	40	0.325±0.001
2	60	0.457±0.001
3	80	0.609±0.002
4	100	0.721±0.002
5	120	0.849±0.003

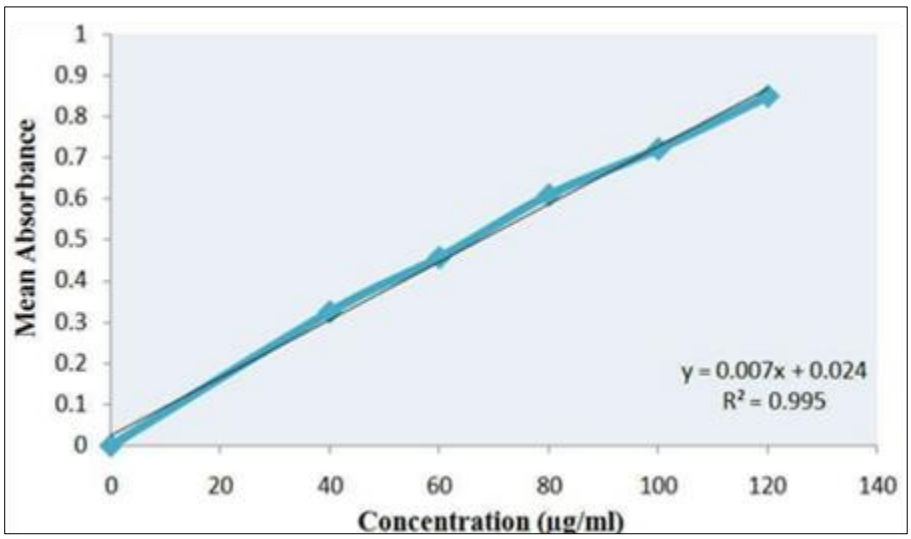

Figure 6 Graph of calibration curve of Atropine

Table 6 Estimation of total alkaloid and flavonoids content of *Cassia angustifolia*

S. No.	Extract	Total alkaloid content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.248	0.614

Table 7 Anti acne activity of standard drug against *Propionibacterium acnes*

S. No.	Name of drug	Zone of inhibition		
		10 µg/ml	20 µg/ml	30 µg/ml
1	Clindamycin	13±0.47	15±0.5	18±0.94

Table 8 Anti acne activity of ethanolic extract of *Cassia angustifolia*

S. No.	Name of microbes	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Propionibacterium acnes*	12±0.74	14±0.57	17±0.74

Figure 7 Photo plates of anti-acne activity of standard drug against *Propionibacterium acnes*

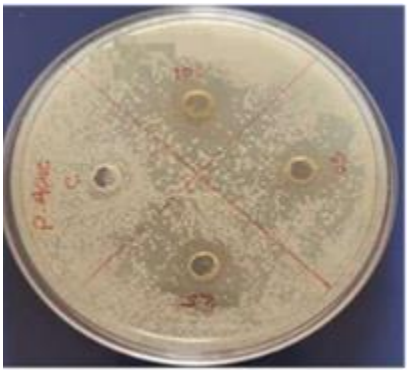

Figure 8 Photo plates of anti-acne activity of ethanolic extract of *Cassia angustifolia* against *Propionibacterium acnes*

Table 9 Protocol study for *in-vivo* anti-acne activity on rats

Groups	**Induction of Acne**	**Treatment**
Control (acne induced)	Heat killed *Propionibacterium acnes*	Vehicle
Treated with hydroalcoholic leaves extract of *Cassia angustifolia*	Heat killed *Propionibacterium acnes*	100 mg/kg p.o.
Treated with hydroalcoholic leaves extract of *Cassia angustifolia*	Heat killed *Propionibacterium acnes*	200 mg/kg p.o.
Treated with Clindamycin	Heat killed *Propionibacterium acnes*	200mg/kg p.o.

Table 10 Effect of Clindamycin (standard) and ethanolic leaves extract of *Cassia angustifolia* induced acne by *Propionibacterium* acnes in rats

Treatment	**Dose**	**Mean thickness ±SEM**				
		Day 2	**Day 4**	**Day 6**	**Day 8**	**Day 10**
Control	140 µg	1.42± 0.50	1.28 ± 0.50	1.25± 0.50	1.27± 0.50	1.27± 0.50
Ethanolic leaves extract of *Cassia angustifolia*	100 mg/kg p.o.	1.38± 0.50*	0.26±0.50*	0.24±0.50*	0.21±0.50*	0.20±0.50*
Ethanolic leaves extract of *Cassia angustifolia*	200 mg/kg p.o.	1.30±0.50**	0.23±0.50**	0.22±0.50**	0.18±0.50***	0.16±0.50***
Clindamycin	200 mg/kg p.o.	1.23±0.50**	0.19±0.50***	0.17±0.50***	0.15±0.50***	0.12±0.50***

Values are expressed as the mean ± SEM of six observations. *** P<0.001 vs. control treatment (One-way ANOVA followed by Dunnett's test)

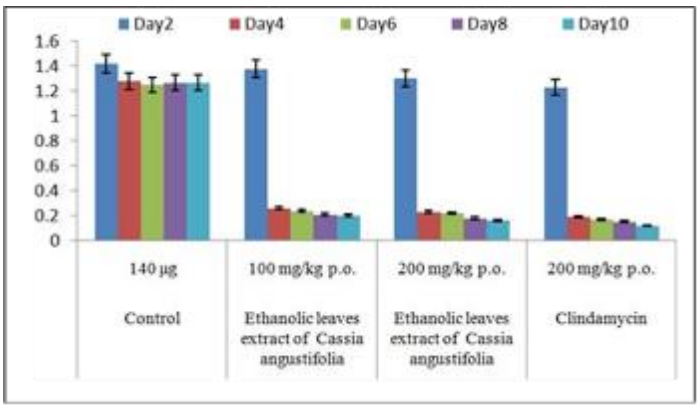

Figure 9 Graph of Effect of Clindamycin (standard) and ethanolic leaves extract of *Cassia angustifolia* induced acne by *Propionibacterium acnes* in rats

4. Conclusion

Cassia angustifolia (Caesalpinaceae) are well known medicinal plant commonly found in India and other tropical countries. Various medicinal properties have been attributed to this plant in the traditional system of Indian medicine.

It is useful in habitual costiveness. It lowers bowels, increases peristaltic movements of the colon by its local action upon the intestinal wall. It is used as expectorant, wound dresser, antidysentric, carminative and laxative. Table no. 7.1 showed the percentage yield of different extract of leaves of *Cassia angustifolia* exhibited percentage yield 1.54% and 6.49% in Petroleum ether and ethanol extract respectively. The preliminary qualitative analysis of the extract showed the initial information of presence/ absence of the various metabolites in the plant extracts. Table no. 7.2 showed the results of phytochemical screening of *Cassia angustifolia* flavonoid, proteins, alkaloids, saponins and diterpenes were detected in ethanol extracted. All phytochemical are absent in pet. ether extract of *Cassia angustifolia* . In thin layer chromatography from the Rf value (0.60) it was confirmed the presence of Quercetin as flavanoids compound in the extract (Table 7.3). Flavonoids are the largest group of polyphenolic compounds having benzo-γ-pyrone structure and ubiquitous in plants. In this study the quantification of flavanoids in the extract of *Cassia angustifolia* was determined by aluminum chloride colorimetric method where quercetin was used as a standard. The present work concluded that *Cassia angustifolia* has significant secondary metabolites which are responsible for the various pharmacological activities. It also proved that a polar solvent ethanol is the most effective solvent to extract the metabolites from the *Cassia angustifolia*. The current results also said that all the extracts posses' detectable amount of alkaloid and flavonoids and antimicrobial activity against *Propionibacterium acnes*. *P. acnes*, as the foremost causative microorganism, M. furfur (yeast), *S. epidermidis*.

S. epidermidis are present in acne lesions. So in the present study hydroalcoholic leaves extract of *Miliusa tomentosa* were selected for the anti-acne activity. The preliminary phytochemical study was carried out according to standard literature. This revealed that they contain various phytoconstituents which can be responsible for the anti-acne activity. The acne-like inflammatory activity was carried out by measuring the ear thickness. Ethanolic leaves extract of *Cassia angustifolia* showed a significant reduction in the ear thickness. It seems that the increased ear thickness and inflammation caused due to various biochemicals, viz. various kinins, histamine and 5-HT is significantly reduced. The presence of various phytoconstituents including the hydroalcoholic leaves extract of *Cassia angustifolia* i.e. flavanoids, phenols showed significant anti-acne properties.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they do not have conflict of interest.

Statement of ethical approval

Ethical approval for the survey was obtained from Institutional Animal Ethics Committee of Technocrates Institute of Technology pharmacy Bhopal M.P. India.

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