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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF POLY-HERBAL FORMULATION FOR ANTI ACNE ACTIVITY

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ABSTRACT

The present study was undertaken anti-bacterial and anti-oxidant activity of methanol extract of *Curcuma longa, Bombax ceiba, Ficus bengalensis, Lens culinaris.* The antibacterial activity, total phenolics and flavinoids were determined by agar-well diffusion method. The zones of inhibition of methanol extracts of *Curcuma longa, Bombax ceiba, Ficus bengalensis* and *Lens culinaris* were found to be 13.375, 11.35, 9.725, 8.625 mm respectively the DMSO did not show any significant antibacterial activity. Anti-oxidant potential was evaluated by using in vitro methods such as 1,1- diphenyl-2- picrylhydrazyl (DPPH), nitro blue tetrazolium chloride test (NBT), OH^-, and O_2^- inhibition free radical assay. The absorbance was measured in UV/vis. Spectroscopy at 590. The total phenolic content (TPC) was expressed as mg/gm of gallic acid equivalent of dry extract sample in order *Bombax ceiba* 27.56 mg/gm > *Ficus bengalensi* 15.65 mg/gm,> *Curcuma longa* 11.27 mg/gm, > *Lens culinaris* 9.68 mg/gm. Total flavonoids content (TFC) was expressed as mg/gm of quercetin in order *Curcuma longa* 21.23 mg/gm> *Bombax ceiba* 18.56 mg/gm> *Ficus bengalensi* 16.56 mg/gm> *Lens culinaris* 12.56 mg/gm. The different fractions significantly ($p < 0.05$) reduce DPPH radical in a dose dependent manner at a concentration for inhibition effect of methanol extract. Suitable ploy herbal formulation is prepared using combination of effacious bioactive and evaluated for anti acne activity.

KEYWORDS: *P. acnes*, anti-bacterial, anti-oxidant, total phenolic content, total flavonoids content, UV- absorption spectra, gel.

INRODUCTION

Acne is an affection that concern 80% of young people in the world with the significant impact on their quality of life.A peak of frequencywas noted in the age group of 21-25 years in the 2 sexes. The prevalence of disease results in 20% of all visits to dermatologist belonging for acne.[1] Acne vulgaris is a most common dermatological disorder of pilosebaceous units and topical therapy is recommended for the management of acne with comedolytic, anti-inflammatory agents ,along with antimicrobials.[2] *Propionibacterium acnes (P. acnes)* play an important role in the pathogenesis of acne inflammation by producing polymorphonuclear leukocyte and monocyte or macrophage to produce proinflammatory mediators. Moreover, *P. acnes* can also induce follicular keratinocytes to release interleukin-1, which causes keratinocytes to proliferate and contributes to the formation of the preclinical micromedo. Therefore, the compounds for targeting acne vulgaris should be able to inhibit *P. acnes.*[3] Many plant species including *Agelica anomala, Garcinia mangostana, Eucommmia ulmoides* etc. have shown anti *Propionibacterium acnes* effect and exhibit potential activity for anti acne treatment.[4] *Curcuma longa,*is widely used as a gold-coloured spice in India act as medicinal herb curcumin a ploy phenolic compound derived has the potential to affect systemic iron metabolism by reducing the level of certian metal ions by chemically scavanging them.[5] The areal root of *Ficus bengalensis L.* contain maximum inhibition of bacterial species[6], *Bombax ceiba* in recent phytochemical studies resulted in the identification of flavinoids, xanthones, coumarin, and other aromatic compounds from this plant[7] and contain anti-inflammatory activities.[8] Seeds of *Lens culinaris,*contain phenolic compounds showed antioxidant and radical-scavenging properties[9], Due to wide therapeutic use of bioactive components used for the treatment of acne this work was aimed to determine and compare the bioactive components i.e. total phenolic compounds, total flavonoids, isolation of different compounds. Moreover antibacterial, anti-inflammatory and antioxidant activities against ***P.acnes***, bacteria involved in acne development. Further combination of the bioactive extracts may have the great potential for treatment of acne in an effective manner without exhibiting side effect or toxicity as indicated by synthetic molecules.

MATERIAL AND METHOD

Preparation of extracts

The plant materials of *Curcuma longa L, Lens culinaris monech , Bombax ceiba L,* and *Ficus benghalensis L.* were collected from Bhopal M.P on December 2013. The materials were authenticated by Dr. Zia Ul Hasan, Department of Botany, Saifia Science College Bhopal

(M.P.) and preserved in the herbarium of the College. Specimen Voucher no: 449/Bot./Saifia/13. was assigned for further reference. The air-dried and powdered defatted marc of the drugs was subjected to extraction with methanol. The crude was soaked in 2.5 L of methanol for 6-7 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a wool plug followed by Whatman filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator.

Quantitative analysis of bioactive compound content in all Methanolic extracts

Total Phenolic content estimation[10,11]

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 25- 150 µg/ml was prepared in methanol.

Preparation of Extract

1gm of dried powder of drug was extracted with 100 ml methanol separately for all extract in 100 ml volumetric flask, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoid content estimation

Principle

Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150 µg/ml were prepared in methanol.

Preparation of extract: 1gm of dried powder of drug was extracted with 100 ml methanol separately for all extract in 100 ml volumetric flask, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of thease extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

Determination of free radical scavenging activity.[12,13]

2.1 *In-Vitro* antioxidant activity

Chemicals

DPPH, NBT, riboflavin and ascorbic acid were purchased from HiMedia Chemicals Ltd. Rest of the chemicals ($FeSO_4$, $FeCl_2$, MeOH, Deionised water, Sodium Salicylate, etc.) were procured from the departmental chemical stores. All the chemicals and reagents used were of highest commercially available purity.

Methods for Evaluating Antioxidant Activity

For the evaluation of the antioxidant activity, *in-vitro* models were screened for the methanol extract for selected materials. The preparations of extract have been already mentioned.

(a) O_2^- scavenger activity assay

Reaction mixture contained 50 mM PBS (pH 7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg/3 ml NBT, added in that sequence. The reaction was started by illumination the reaction the reaction mixture with different concentration of samples. Immediately after illumination, the absorbance was measured in UV/vis. Spectroscopy at 590 nm. Ascorbic acid was used as positive control. Readings were taken at 15, 30 and 45 min.

Preparation of reaction mixtures and measurement of scavenging

Methodology - 100 µl riboflavin solutions, 200 µl EDTA solutions, 200 µl ethanol and 100 µl of NBT solution was mixed in a Borosilicate test tube and the reaction mixture was diluted up to 3 ml with PBS and the whole reaction mixture was illuminated for 15 min. Just after illumination the reading were taken at 590 nm using PBS as a blank. This was taken as control reading.

100 µl of test solutions i.e. methanol extract, 100 µl riboflavin solution, 200 µl EDTA solution, 200 µl ethanol and 100 µl of NBT solution was mixed in a Borosilicate test tube and the reaction mixture was diluted up to 3 ml with PBS and the whole reaction mixture was illuminated for 15 min. Just after illumination the reading were taken at 590 nm using PBS as a blank. This was taken as control reading. Different reaction mixtures were prepared for each test samples.

*** Note**: Methanol extracts 10 mg/ml were used for this study.

Percent reduction of O_2^- generation was calculated as

$$\% \text{ reduction} = \frac{\text{Control abs.} - \text{Test abs.}}{\text{Control abs.}} \times 100$$

(b)Determination of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

Each methanolic extract (0–20 mg/ml) dissolved in deionized water (2 ml), was mixed with 2 ml of methanol solution containing DPPH radicals, resulting in a final concentration of 0.1 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank. Ascorbic acid was used as the positive control.

The percentage scavenging was calculated as:

$$\% \text{ I scavenging} = \frac{\text{Control abs.} - \text{Test abs.}}{\text{Control abs.}} \times 100$$

(c) OH^- scavenging assay

OH^- scavenging ability was measured according to a literature procedure with a few modifications. OH^- radicals were generated from $FeSO_4$ and H_2O_2, and detected by their ability to hydroxylate salicylate. The reaction mixture (3 ml) contained 1 ml $FeSO_4$ (1.5 mM), 0.7 ml H_2O_2 (6 mM), 0.3 ml sodium salicylate (20 mM) and varying concentrations of methanolic extract. After incubation for 1 h at 37^{o}C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as.

The percentage scavenging was calculated as:

$$\% \text{ I scavenging} = \frac{\text{Control abs.} - \text{Test abs.}}{\text{Control abs.}} \times 100$$

Determination of antimicrobial activity

Bacterial Stock Culture

A loopful of bacterial strain was transferred to nutrient medium and incubated overnight at 37°C. The number of colony forming unit were found to be 10^3 per ml.

Preparation of Sample

Solutions of methanol extracts of selected plants were prepared using 10 mg of each in 10 ml of dimethyl sulfoxide (DMSO). Tetracycline (10 mg/ml) was used as a positive and DMSO as a negative control.

Agar Diffusion Method [3]

The antibacterial activity of selected plants extracts were determined by modified agar well diffusion method. The agar diffusion test was used to investigate antibacterial effects of methanol extracts of selected plants. In this method plates containing 10 ml of agar media and Sabouraud agar medium were over laid with 10 ml of inoculated stock solution of *P. acnes* bacteria. Equidistant holes were made in the agar. A sterile 8 mm borer was used to cut four wells of equidistance in each of plates. 1ml volume of selected plant extracts, and standard drug (10 mg/ml) was pipetting into the agar the wells at randomly. Standard compound tetracycline (10 mg/ml) was used as positive control and the negative control was DMSO. After 24 h and 3-5 days incubation the diameter of the inhibition zones, (no growth) around the holes in the bacterial turf were measured. A positive result was defined as an inhibition zone (halo size) of 9 mm or more around the holes, therefore indicating the presence of antibacterial substance in the tested sample. The experiments were repeated four times.

RESULTS AND DISCUSSION

1.6 Total Phenolic content estimation (TPC). [14]

The content of total phenolic compounds (TPC) and to total tannin content was expressed as mg/gm of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.002X+ 0.025, R^2= 0.980,** where × is the absorbance and y is the tannic acid equivalent (GAE).

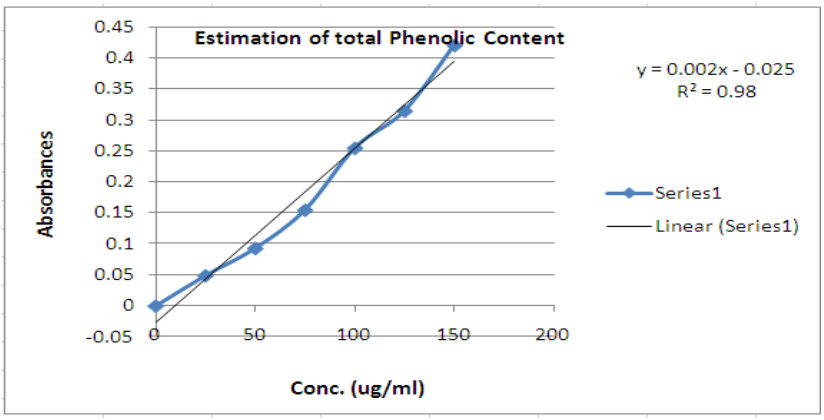

Fig. 1 Total Phenolic content estimation (TP

Table 1: Total Phenolic content estimation (TPC)

S.No.	Conc.	Absorbance
0	0	0
1	25	0.049
2	50	0.093
3	75	0.155
4	100	0.255
5	125	0.315
6	150	0.421

1.7 Total Flavonoids content estimation (TFC).[15]

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: **Y=0.004 X+0.001, R^2=0.996**, where X is the absorbance and Y is the quercetin equivalent (QE).

Table 2: Total Flavonoids content estimation (TFC)

S.No.	Conc.	Absorbance
0	0	0
1	25	0.119
2	50	0.195
3	75	0.297
4	100	0.387
5	125	0.517
6	150	0.626

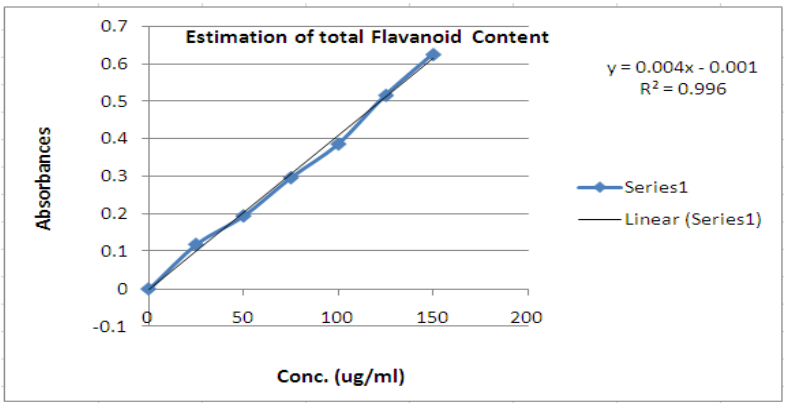

Fig. 2 Total flavonoids content estimation (TFC)

Table 3: Estimation of Total phenolics and Total flavonoids content

S. No	Methanolic Extracts	Total phenolic Content (mg/gm)	Total flavonoids content (mg/gm)
1.	*Curcuma longa*	11.27	21.23
2.	*Bombax ceiba*	27.56	18.56
3.	*Ficus bengalensis*	15.65	16.56
4.	*Lens culinaris*	9.68	12.56

RESULTS AND DISCUSSION

The antioxidant activity of methanol extract of selected plant was evaluated using *in vitro* models.

Inhibition of superoxide anion radical

Superoxide radical is very harmful to cellular components as a precursor of ROS. Methanol extract inhibited O_2^- radical generated in riboflavin-NBT light system. The results were found statistically significant shown in (table. 4) and (fig. 3).

5. 2 Inhibition of DPPH radical

The antioxidant reacts with DPPH radical (purple colour) and converts it into a colourless α-α-diphenyl-β-picrylhydrazyl. The amount of DPPH reduced could be quantified by measuring decrease in absorbance at 517 nm. The different fractions significantly ($p < 0.05$) reduce DPPH radical in a dose dependent manner at a concentration. The inhibition effect of methanolic extract was show in (table 5& 7), (fig. 4).

Inhibition of OH^- radical

The highly reactive OH^- radical can cause oxidative damage to DNA, lipid and proteins .The effect of extract on the inhibition of free radical mediated deoxyribose damage was assessed by means of iron (II) dependent DNA damage assay. The Fenton reaction generate OH^- radical, which degrade deoxyribose sugar of DNA using Fe^{2+} salt as an important catalytic component. The inhibition effect of methanolic extract was show in (table 6,8) and (fig. 5).

Table 5 : % Inhibition by DPPH scavenging activity

Group	Scavenging agent	%Inhibition					
		2 mg/ml	6 mg/ml	8 mg/ml	10 mg/ml	12 mg/ml	20 mg/ml
I.	Control	-	-	-	-	-	-
II.	Methanolic extract of *Curcuma longa*	41.0 %	41.3 %	42.7 %	43.0 %	44.7 %	49.9 %
III.	Methanolic extract of *Bombax ceiba*	58.0 %	59.1 %	61.8 %	65.7 %	65.9 %	73.8 %
IV.	Methanolic extract of *Ficus benghalensis*	44.2%	44.7 %	45.3 %	49.5 %	53.3 %	71.0 %
V.	Methanolic extract of *Lens culinaris*	36.7 %	37.9 %	40.5 %	41.4 %	41.9 %	42.4%
VI.	Std.	80.0 %	82.0 %	87.2 %	87.7 %	89.7%	90.4 %

Data are expressed as mean± S.E.M. ($n = 6$), $^{**}p < 0.01$; ns: not significant vs. control.

Table 6: % inhibition by OH^- scavenging activity

Group no.	Scavenging agent	2 mg/ml	6 mg/ml	8 mg/ml	10 mg/ml	12 mg/ml	20 mg/ml
I.	Control	-	-	-	-	-	-
II.	Methanolic extract of *Curcuma longa*	39.3 %	40.3 %	40.7 %	42.1 %	44.4 %	48.0 %
III.	Methanolic extract of *Bombax ceiba*	68.0 %	69.1 %	69.8 %	71.7 %	71.0 %	77.7 %
IV.	Methanolic extract of *Ficus benghalensis*	40.0 %	40.5 %	43.3 %	43.7 %	53.9 %	67.3 %
V.	Methanolic extract of *Lens culinaris*	39.3 %	40.3 %	40.7 %	42.1 %	44.4 %	48.0 %
VI.	Std.	77.7 %	79.0 %	80.2 %	81.7 %	83.7 %	85.0 %

Table 4 : Superoxide anion free radical scavenging activity of chosen drugs

Time (min)	Control	MCL	MBC	MFB	MLC	Standard
15	-	49.9±0.86	78.0±1.38	62.9±1.08	33.0±0.67	89.0±1.76
30	-	47.2±0.84	68.8±1.23	54.2±1.02	32.7±0.66	84.2±1.68
45	-	42.5±0.79	67.0±1.18	52.5±0.97	32.0±0.62	82.7±1.61

Table 7 : % inhibition of free radical scavenging activity of chosen drugs by DPPH method

Conc. (mg/ml)	Control	MCL	MBC	MFB	MLC	Standard
2	-	41.0±0.79	58.0±1.18	44.2±0.84	36.7±0.67	80.0±1.46
6	-	41.3±0.84	59.1±1.20	44.7±0.88	37.9±0.69	82.0±1.57
8	-	42.7±0.94	61.8±1.24	45.3±0.92	40.5±.72	87.2±1.59
10	-	43.0±0.74	65.7±1.28	49.5±1.04	41.4±0.76	87.7±1.62
12	-	44.7±0.68	65.9±1.34	53.3±1.14	41.9±0.78	89.7±1.65
20	-	49.9±0.72.	73.8±1.44	71.0±1.32	42.4±0.82	90.4±1.74
10	-	43.0±0.74	65.7±1.28	49.5±1.04	41.4±0.76	87.7±1.62

Table 8 : % inhibition of OH^- free radical scavenging activity of chosen drugs

Conc. (mg/ml)	Control	MCL	MBC	MFB	MLC	Standard
2	-	41.0±0.79	58.0±1.18	44.2±0.84	36.7±0.67	80.0±1.46
6	-	41.3±0.84	59.1±1.20	44.7±0.88	37.9±0.69	82.0±1.57
8	-	42.7±0.94	61.8±1.24	45.3±0.92	40.5±.72	87.2±1.59
10	-	43.0±0.74	65.7±1.28	49.5±1.04	41.4±0.76	87.7±1.62
12	-	44.7±0.68	65.9±1.34	53.3±1.14	41.9±0.78	89.7±1.65
20	-	49.9±0.72.	73.8±1.44	71.0±1.32	42.4±0.82	90.4±1.74
10	-	43.0±0.74	65.7±1.28	49.5±1.04	41.4±0.76	87.7±1.62

Data are expressed as mean± S.E.M. (n = 3)

MCL =	Methanol extract of *Curcuma longa*
MBC =	Methanol extract of *Bombax ceiba*
MFB =	Methanol extract of *Ficus bengalensis*
MLC =	Methanol extract of *Lens culinaris*

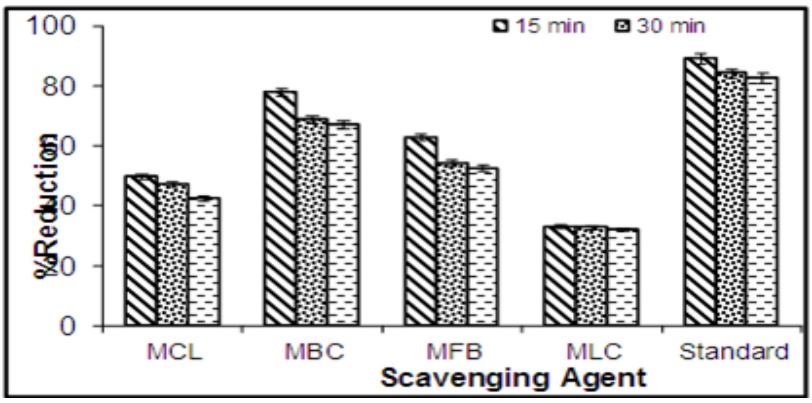

Fig. 3: Superoxide anion free radical scavenging activity of chosen drugs.

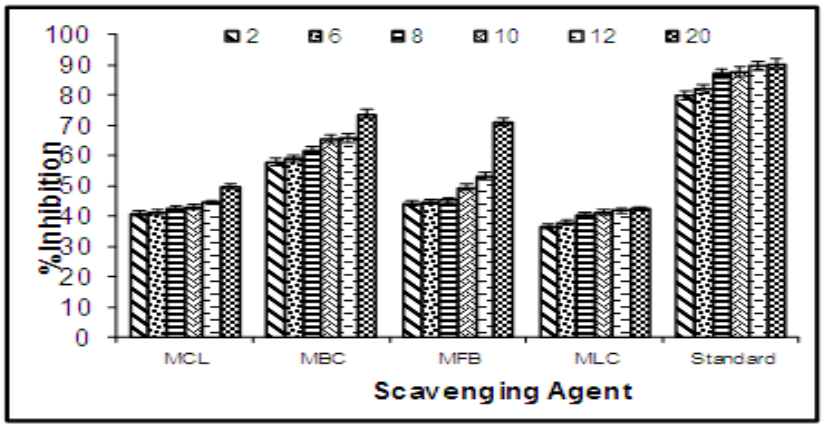

Fig. 4 : % inhibition of free radical scavenging activity of chosen drugs by DPPH method in different concentration.

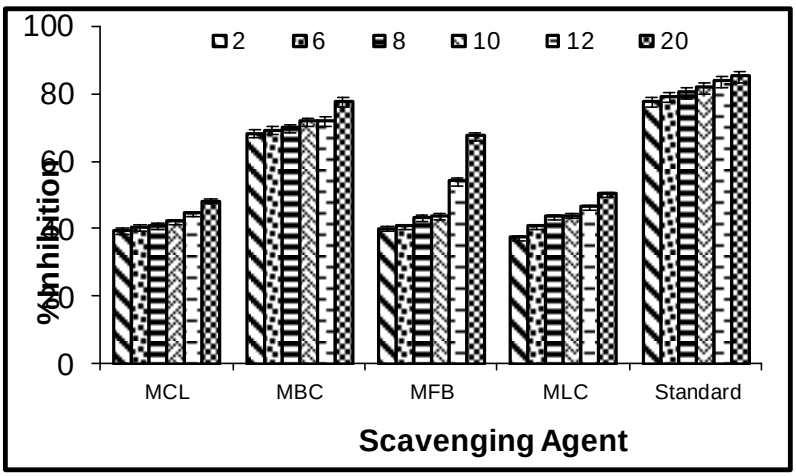

Fig. 5 : % inhibition of OH^- free radical scavenging activity of chosen drugs in different concentrations

RESULTS AND DISCUSSION

From the above investigations it was revealed that methanol extract of *Curcuma longa, Lens culinaris, Bombax ceiba,* and *Ficus bengalensis* was bring into being to scavenge DPPH, OH, and O_2^- inhibition. Methanol extract of all selected plants shown strong antioxidant activity in compression of selected plant. This methanol extract of *Bombax ceiba* has shown strong antioxidant activity due to presence of terpenoids and flavonoids therein as revealed by the quenching of the DPPH radical. The antioxidant activity can be endorsed due to the existence of different fractions, which were confirmed by phytochemical tests. The positive free radical scavenging property of *Bombax ceiba* is valuable therapeutic potential. The facts obtained in the current study imply that methanol extract of selected plants have persuasive antioxidant activity in a concentration dependent manner.

RESULTS AND DISCUSSION

The data obtained from agar well diffusion method using different methanol extracts and standard were shown in Table:9. The zones of inhibition of methanol extracts of *Curcuma longa*, *Bombax ceiba*, *Ficus bengalensis* and *Lens culinaris* were found to be 13.375, 11.35, 9.725, 8.625 mm respectively. The results of this investigation showed that all methanol extracts had inhibitory effect on the *P. acnes*.

The present research work deals with the antibacterial activity against *P. acnes* bacteria of selected plants. The Zones of inhibitions for the antibacterial activity were compared with the standard tetracycline with the methanol extracts. The results of present investigation showed that methanolic extracts of *curcuma longa* and *Ficus bengalensis* had better inhibitory effect against *P. acnes* bacteria compare to other extracts. However, the activity of the standard tetracycline shown best activity in comparisons with extract of selected plants. DMSO did not show any significant antibacterial activity.

Table 9: Antibacterial activities of selected methanol extracts

S. No.	Treatment	Zones of inhibition in mm				Mean (n= 4)
		1	2	3	4	
1.	Tetracycline (10 mg/ml)	20	19	18.2	17.9	18.775
2.	Methanol extract of *Curcuma longa* (10 mg/ml)	13.2	13.8	13.7	12.8	13.375
3.	Methanol extract of *Ficus bengalensis* (10 mg/ml)	11.7	11.4	10.5	11.8	11.35
4.	Methanol extract of *Bombax ceiba* (10 mg/ml)	9.9	9.7	9.5	9.8	9.725

5.	Methanol extract of *Lens culinaris* (10 mg/ml)	8.2	8.7	8.3	9.3	8.625
6.	DMSO	---	---	---	---	---

PREPARATION OF THE COMBINATION OF SELECTED HERBS

On the bases of results of antibacterial and antioxidant activity of individual selected plant extract (methanol) carried out reveals that methanol extracts of *Curcuma longa* and *Ficus bengalensis* have shown good antibacterial activity in comparisons to other methanol extract. The methanol extracts of *Bombax ceiba* had exert the best antioxidant activity in comparisons to other extracts. It has also provided antibacterial activity against *P. acne* bacteria.

After the study of antioxidant, antibacterial activity of methanol extract of selected plants, Methanol extract of *Curcuma longa, Ficus bengalensis* and *Bombax ceiba* was selected for preparation of combination.

It was believed that the combination effect of these drugs may give more significant effect as, antibacterial and antioxidant activity. Hence it was decided to carry out the combination study of these drug samples.

The combinations of different plants methanol extract (*Curcuma longa*, *Ficus benghalensis*, *Bombax ceiba*) were prepared in 500 µl ethanol for evaluation of their antibacterial activity.[16] Show in (table. 10).

Table 10: Combination of Methanol extracts

Combination	Methanol Extract of Plant			
	***Curcuma longa* (mg)**	***Ficus benghalensis* (mg)**	***Bombax ceiba* (mg)**	**Total Quantity (mg)**
1	250	------	250	500
2	250	250	------	500
3	------	250	250	500
4	200	200	100	500

The antibacterial activity of different combinations was determined by modified agar well diffusion method. The agar diffusion test was used to investigate antibacterial effects of combinations of selected plants. In this method plates containing 10 ml of agar media and Sabouraud's agar medium were over laid with 10 ml of inoculated stock solution of *P. acne* bacteria. Equidistant holes were made in the agar. A sterile 8 mm borer was used to cut four wells of equidistance in each of plates. 1ml volume of selected combinations (10 mg/ml) was pipetting into the agar the wells at randomly. Standard compound tetracycline (10 mg/ml)

was used as positive control and the negative control was DMSO. After 24 h incubation the diameter of the inhibition zones, (no growth) around the holes in the bacterial lawn were measured. A positive result was defined as an inhibition zone (halo size) of 9 mm or more around the holes, therefore indicating the presence of antibacterial substance in the tested sample.[17] The experiments were repeated four times.

Table 11: Antibacterial activity of selected Methanol extracts

S.No.	Treatment	Zones of inhibition in mm				Mean ±SEM (n= 4)
		1	2	3	4	
1.	Indomethacin (10 mg/ml)	20	19	18.2	17.9	18.775
2.	Combination 1	13.2	13.8	13.7	13.4.	13.566
3.	Combination 2	15.7	14.8	15.5	15.1	15.275
4.	Combination 3	9.9	9.7	9.5	9.8	9.725
5.	Combination 4	19.7	20.2	19.2	18.8	19.475
6.	DMSO	---	---	---	---	---

4.3.4 Statistical Treatment of Data

All the statistical analysis was performed by statistical treatment mean ±SEM (n= 4) [11].

The antibacterial activity of an combination of methanolic extract of *Curcuma longa*, *Ficus benghalensis* and *Bombax ceiba* was evaluated in the present study. The combination demonstrated antibacterial activity against *P. acnes* strains tested in this study. The results of present investigation showed that combination 4 (methanolic extracts *curcuma longa, Ficus benghalensis and Bombax ceiba*) had better inhibitory effect 19.475 against *P. acnes* bacteria compare to other combinations. However, combination1, 2 and 3 shown zones of inhibition were 13.566, 15.275 and 9.725 respectively. DMSO did not show any significant antibacterial activity. These results suggested that the herbal combination 4 may be a more effective against *P. acnes*. Shown in (table.11).

RESULT AND DISCUSSION

The methanolic extract of *Curcuma longa, Bombax ceiba, Ficus bengalensis, Lens culinaris* consists considerable amount to total phenolic and flavonoid contents. The methanol extracts of *Bombax ceiba* had exert the best antioxidant activity in comparisons to other extracts. And combination of selected methanol extract had better inhibitory activity compared to methanol extract of single plants against bacterial strain *P. acne.*

CONCLUSION

The study proves that the antibacterial and antioxidant properties of the chosen drugs and at the end were suitable in developing a Gel formulation from utilize the bio-actives of extracts as combination 4 comprising a methanol extracts of (i) *Curcuma longa* (ii) *Ficus bengalensis* (iii) *Bombax ceiba* for the effective management of the Acne.

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