Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

# IN VITRO ANTIBACTERIAL AND ANTIOXIDANT POTENTIAL OF MEDICINAL PLANTS USED IN THE TREATMENT OF ACNE

**PRATIBHA NAND [1], SUSHMA DRABU[1], RAJINDER K GUPTA[2*]**

[1]Maharaja Surajmal Institute of Pharmacy, Janakpuri, [2]University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi, India. Email: rkg67ap@yahoo.com

*Received: 12 Feb 2011, Revised and Accepted: 18 May 2011*

**ABSTRACT**

In the present study, petroleum ether, dichloromethane and methanolic extracts of three medicinal plants namely *Camellia sinensis*, *Glycyrrhiza glabra* and *Calendula officinalis* were analyzed for their antibacterial and antioxidant activity in different test systems. Highest zone of inhibition (≥15) mm was observed with methanolic extract of *Camellia sinensis* using disc diffusion method. Phytochemical analysis indicated that amongst nine test extracts, methanolic extract of *Camellia sinensis* had the highest total phenolic content (104.93 ± 1.630 mg GAE /g). Flavonoid (115.503 ± 2.984 mg RuE /g dry extract) and flavonol content (574.446 ± 7.94mg RuE /g dry extract) of methanolic extract of *Glycyrrhiza glabra* was also found to be superior among all the extracts. Antioxidant assays revealed highest DPPH scavenging ($IC_{50}$=44.03 ± 1.784 µg/ml) and metal chelating ($IC_{50}$=234.64 ± 5.467 µg/ml) effect in methanolic extracts of *Camellia sinensis*. Similarly, methanolic extract of *Glycyrrhiza glabra* exhibited highest radical scavenging activity ($IC_{50}$=21.37 ± 1.422 µg/ml) when reacted with the $ABTS^{\cdot+}$.

**Keywords**: Acne, Free radicals, Medicinal plants, Total phenolic, Total flavonoid, Total flavonol, DPPH, ABTS, Metal chelating.

## INTRODUCTION

Acne vulgaris is the most common skin disorder of pilosebaceous unit, generally characterized by formation of seborrhea, comedone, inflammatory lesions and presence of bacteria namely *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes* in the follicular canal[1]. *Propionibacterium acnes* evokes mild local inflammation by producing neutrophil chemotactic factors. Consequently, neutrophils get attracted to the acne lesions and constantly release inflammatory mediators such as reactive oxygen species (ROS) [2]. Although oxygen is an important component for human beings, yet it can produce various ROS such as superoxide anion, hydrogen peroxide and hydroxyl radicals etc. Furthermore, ROS play a critical role in irritation and disruption of the integrity of the follicular epithelium and are responsible for the progression of inflammatory acne[3]. These toxic ROS can also act as messengers in the induction of several biological responses such as NF-kB, AP-1 and the generation of cytokines. These radicals are formed with the reduction of oxygen to water. Normally, the production of these radicals is slow and they are removed naturally by antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glucose-6-phosphate dehydrogenase (G6PD) existing in the cell, but due to the depletion of immune system and natural antioxidants in different ailments, it becomes necessary to use antioxidants as free radical scavengers for the removal of ROS to reduce cell damage that occurs during acne inflammation[4]. Drugs like tetracycline, erythromycin, minocycline and metronidazole are gaining more importance and are preferred over antibiotics [5] because of their antioxidant effect. Moreover, antibiotic resistant *P. acnes* has now become a critical problem worldwide[6]. Hence Antioxidants are extensively studied for their capacity to protect organism and cell from damage that are induced by oxidative stress[7]. There are a number of synthetic antioxidants like butylated hydroxy anisole, butylated hydroxy toluene, propyl gallate and gallic acid esters which are available but are suspected to cause negative health effects and are also unstable at elevated temperatures[8]. Hence, the objective of our research work was to investigate antibacterial and antioxidant potential of the following three medicinal plants viz: *Camellia sinensis*[9], *Glycyrrhiza glabra*[10, 11] and *Calendula officinalis*[12, 13].

## MATERIALS AND METHODS

### Plant material, chemicals and reagents

Fresh and dried plant materials were collected from medicinal gardens and authorized herbal stores in Delhi. Their botanical identities were determined and authenticated at the National Institute of Science Communication and Information Resources, New Delhi, India vide voucher specimen (NISCAIR/RHM/consult/2007-08/936/120) and (NISCAIR/RHM/consult/2008-09/978/09). Clindamycin phosphate was procured from Sri Ram Institute of Industrial Research, New Delhi. All other chemicals used were of analytical grade and obtained from either Sigma-Aldrich or Merck.

### Preparation of extracts and phytochemical screening

The air dried leaves, flowers, stolons, roots and seeds were pulverized and passed through sieve #10 and were used for extraction in soxhlet apparatus at room temperature. Sequential extraction of 200 g was carried out with solvents of increasing polarity i.e. petroleum ether (PE), dichloromethane (DCM) and methanol (ME) and the extracts were abbreviated as: *Camellia sinensis* (CSPE, CSDCM, CSME), *Glycyrrhiza glabra* (GGPE, GGDCM, GGME) and *Calendula officinalis* (COPE, CODCM, COME). The extracts were evaporated under vacuum conditions using a rotary evaporator and stored at°C in air tight containers for further studies. The percentage yield was recorded. Preliminary phytochemical screening of the test extracts was carried out[14].

### Antimicrobial screening of extracts

### Microorganisms and media

Aerobic bacteria: *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 2639) and anaerobic bacteria: *Propionibacterium acnes* (MTCC *1951) were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh. Fresh cultures of the isolates of aerobic and anaerobic bacteria were suspended in nutrient broth and reinforced clostridium medium respectively. *S. aureus* and *S. epidermidis* cultures were incubated for 24 h at 37°C and 30°C, respectively. *P. acnes* culture was incubated in an anaerobic chamber at 37°C consisting of 10% $CO_2$, 10% $H_2$ and 80% $N_2$ for 48 h.

### Disc diffusion method

Antibacterial activity of extracts was tested using agar disc diffusion method[15]. 100 µl of fresh culture suspension of test bacteria was evenly spread on nutrient agar and reinforced clostridial agar plates. The concentration of cultures was $5x10^5$ CFU/ml. For screening, 6 mm diameter filter paper disc, impregnated with 20 µl of extract solution equivalent to 0.2 mg of extract, was placed on the surface of inoculated media agar plates. Incubation was done at 37°C or 30°C for 24 h and 48 h depending upon the type of bacteria under optimum conditions. Clear zones of inhibition were measured in mm and Clindamycin (10 µg/disc) was used as positive control.

**Phytochemical analysis**

**Determination of total phenolic content**

The total phenolic content of the nine extracts was determined using McDonald method with modifications[16]. 100 µl of the diluted extracts containing 500 µg and standard phenolic compound gallic acid (10-50 µg/ml) were mixed separately with (62.5 µl) Folin-Ciocalteu reagent and diluted with 0.287 ml distilled water and 0.375 ml of 20% aqueous $Na_2CO_3$. The mixtures were then allowed to stand for 2 h and the total phenolic content was determined using spectrophotometer at 765 nm. The concentrations of the total phenolic compounds were calculated using the equation (y = 0.02769× + 0.0103: $r^2$ = 0.9984) and the total phenolic content was expressed as mg of Gallic acid equivalents (GAE) /g of dried extract.

**Determination of total flavonoid content**

The total flavonoid content of test extracts was determined using existing Chang et al. method with some modifications[17]. 100 µl of the extracts containing 500 µg was mixed with 300 µl of distilled water and 30 µl of 5% $NaNO_2$. The mixture was kept at room temperature for 5 min followed by addition of 30 µl of 10% $AlCl_3$, 0.2 ml of 1 mM NaOH and distilled water. The absorbance of the reaction mixture was measured at 415 nm with UV spectrophotometer. The concentration of the flavonoid compounds was calculated using the equation (y=0.01083x -0.00476: $r^2$ = 0.9945) obtained from the rutin (20-100 µg/ml) calibration curve and the flavonoid content was expressed as mg of rutin equivalents (RuE) per g of dried extract.

**Determination of total flavonol content**

Total flavonol content of the test samples was estimated using Miliauskas et al. modified method[18]. Calibration curve of the standard was prepared by mixing methanolic rutin solution (20-100 µg/ml) with 1 ml of 2% $AlCl_3$ and 3 ml of 5% sodium acetate. The absorption was read after 2.5 hrs at 20°C at 440 nm. The same procedure was carried out with 100 µl of plant extract (10 mg/ml) containing 500 µg instead of rutin solution. The flavonol content was expressed as mg of rutin equivalents (RuE) per g of dried extract using the equation (y=0.00482x-0.00445: $r^2$ = 0.9961).

**Antioxidant capacity**

**DPPH Assay**

The free radical scavenging activity was estimated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay using Blois method with some modifications[19]. The reaction mixture contained 100 µl of test extracts (100-500 µg/ml) and 1 ml of methanolic solution of 0.1 mM DPPH radical. The mixture was then vigorously shaken and incubated at 37°C for 30 min. The absorbance was measured at 517 nm using ascorbic acid (100-500 µg/ml) as positive control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity which was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = 100 \times (A_o - A_1)/(A_o)$$

where $A_o$ is the absorbance of the control reaction and $A_1$ is the absorbance of reaction mixture containing DPPH and extract at 517 nm. The antioxidant activity of the extract was expressed as $IC_{50}$ value which is defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50%. This was obtained from linear regression analysis.

**$ABTS^{\bullet+}$ radical cation decolorization assay**

$ABTS^{\bullet+}$ radical scavenging activity of the test samples was measured using Re et al. method with minor modifications[20]. It measures the reduction of the ABTS radical cation by an antioxidant. ABTS radical cation (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate in 100 mM phosphate buffer solution (pH 7.4) and keeping the mixture in a dark place at room temperature for 12- 24 h before use. This solution was further diluted with 100 mM phosphate buffer solution (pH 7.4) to give an absorbance of 0.700 ± 0.02 at 734 nm. For the study of radical scavenging activity, 900 µl of ABTS was added to 100 µl of various concentrations (100-500 µg/ml) of the extracts and ascorbic acid. The reaction mixture was then incubated for 20 min and the absorbance was measured at 734 nm using methanol as a blank. $ABTS^{\bullet+}$ radical scavenging activity was calculated using the same formula as mentioned in DPPH assay.

**Metal chelating effect on ferrous ion**

The chelation of ferrous ions by test extracts was measured using the Dinis et al. modified method[21]. To 1 ml of test extracts (100-500 µg/ml) and 50 µl of 2 mM $FeCl_2$ in the reaction mixture, 0.2 ml of 5 mM ferrozine solution was added to initiate the reaction. The reaction mixture was then vigorously shaken and left untouched at room temperature for 10 min and then the absorbance of the mixture was measured at 562 nm. The chelating activity of the extracts was evaluated using $Na_2EDTA$ as standard. The percentage inhibition of ferrozine-$Fe^{2+}$ complex formation was calculated using the same formula as mentioned in DPPH scavenging activity.

**Statistical analysis**

All the samples were run in triplicate and the mean values were used for result analysis. The statistical significance between the antioxidant activity of extracts and standards was evaluated using SPSS version 10.0.1 and comparison was made using Mann-Whitney U test.

**RESULTS AND DISCUSSION**

**Antimicrobial screening**

*In vitro* antimicrobial screening using clindamycin phosphate as a positive control clearly indicated that CSME, GGME and COME show promising antimicrobial activity against all the three organisms. It was observed that all the extracts of *C. sinensis* and *G. glabra* show significant antimicrobial activity against test organisms except GGPE which did not exhibit antimicrobial activity against *S. epidermidis*. CODCM and COME were found to be significantly active against *S. epidermidis,* but did not show inhibitory activity against *S. aureus* and *P. acnes*. Highest zone of inhibition, 17.8 ± 0.016 mm, was observed for CSME against *S. epidermidis* (Table 1).

**Table 1: Antimicrobial screening of plants against *S. aureus* (MTCC 96), *S. epidermidis* MTCC 2639) and *P. acnes* (MTCC *1951) using disc diffusion method**

| **Zone of inhibition of extracts in mm** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ***S. aureus**** | | | ***S. epidermidis**** | | | ***P. acnes**** | | |
| | **PE** | **DCM** | **ME** | **PE** | **DCM** | **ME** | **PE** | **DCM** | **ME** |
| *Camellia* | 7.5 | 9.0 | 14.4 | 7.4 | 10.0 | 17.8 | 7.6 | 7.8 | 13.8 |
| *sinensis* (Le) | ±0.28 | ±0.06 | ±0.27 | ±0.05 | ±0.06 | ±0.16 | ±0.18 | ±0.1 | ±0.2 |
| *Glycyrrhiza* | 7.66 | 9.06 | 11.5 | NA | 8.0 | 11.8 | 7.1 | 13 | 13.9 |
| *glabra* (R&S) | ±0.16 | ±0.06 | ±0.28 | | ±0.12 | ±0.15 | ±0.05 | ±0.05 | ±0.15 |
| *Calendula* | NA | 9.06 | NA | NA | 9.06 | 11.8 | 9.13 | NA | 12.4 |
| *officinalis*(Fl) | | ±0.12 | | | ±0.17 | ±0.15 | ±0.08 | | ±0.18 |
| Clindamycin | 14.94 | | | 18 | | | 18 | | |
| phosphate | ±0.08 | | | ±0.11 | | | ±0.05 | | |

Le = leaves; R & St = roots and stolons; Fl = flowers; PE = petroleum ether extract; DCM = dichloromethane extract; ME = methanolic extract; NA = No antibacterial activity. Values are Mean ± SEM (mm) of three measurements; *P< 0.05.

There is lack of reports revealing evidence for the antimicrobial activity of these medicinal plants against acne causing bacteria, still our findings could be based on the antibacterial action of epigallocatechin gallate and epicatechin in *C. sinensis*[22]. Antibacterial and anti-inflammatory effect of *G. glabra* is attributed to the presence of glycyrrhizin and its hydrolysis product, glycyrrhetinic acid[23]. Presence of triterpenoids in *C. officinalis* is known to provide anti-inflammatory activity. It has also been reported that esters of faradiol-3-myristic acid, faradiol-3-palmitic acid and 4-taraxasterol are the three most active compounds to reduce edema and the flavonoid, kaempferol, has antibacterial potential against *P. acnes*[24].

**Phytochemical analysis**

**Total phenolic, flavonoid and flavonol content**

Phenols are the simplest bioactive phytochemicals possessing free radical scavenging ability due to the presence of hydroxyl groups. The site and the number of hydroxyl groups present are related to their relative toxicity to microorganisms, showing that increased hydroxylation results in increased toxicity. The results of the present study clearly indicate (Figure 1) that amongst all the test extracts, CSME shows the highest amount of GAE of phenolic compounds (104.934 ± 1.630 mg/g).

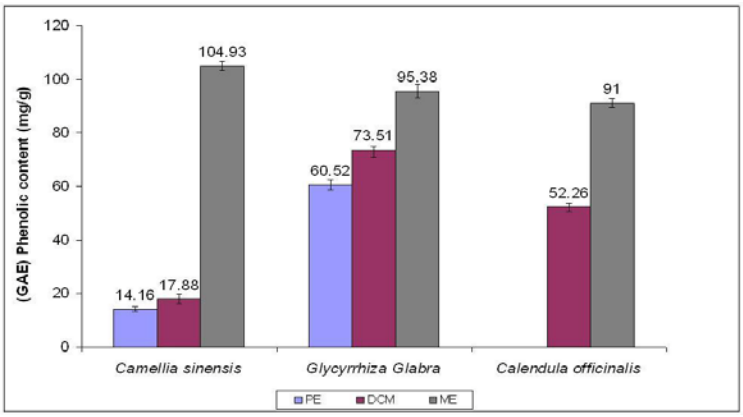


**Fig. 1: Total phenolic content of test extracts**

COPE did not show any phenolic content. Flavonoid and flavonol are polyphenolic compounds which play an important role in stabilizing lipid oxidation and are also associated with antioxidative action[25]. Highest and lowest flavonoid content was observed in GGME (115.503 ± 2.984 mg/g) and COPE (2.488 ± 1.659 mg/g) respectively (Figure 2).

Furthermore, flavonol content of the extracts was between (574.443 ± 7.937 and 15.532 ± 1.229 mg/g). Highest flavonol content was observed in GGME (574.443 ± 1.937 mg/g). COPE didn't show any flavonol content (Figure 3). The total phenolic, flavonoid and flavonol content of extracts was found to be statistically significant (P< 0.05).

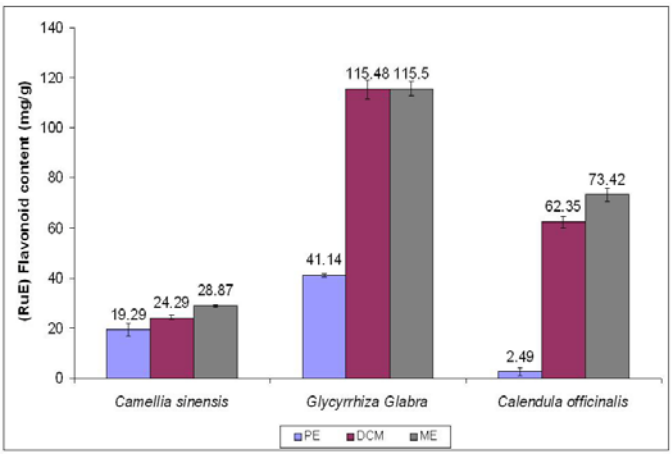


**Fig. 2: Total flavonoid content of test extracts**

Results from this study support the previous findings as *Camellia sinensis is* well known to be rich in polyphenolic content and possesses antioxidant activity. The constituents of *C. sinensis* include large amounts of (-)-epigallocatechin, (-)-epicatechin, (+)-gallocatechin, (+)-catechin and their derivatives, rutin and myricetin, which have been shown to possess high antioxidant and free radical scavenging activity and positive effect on human health[26,27]. Even Licorice flavonoid constituents are known to have free radical scavenging effect[28] and antioxidant potential[29]. Licorice flavonoid constituents mainly consist of flavones, flavonols, isoflavones, chalcones, dihydroflavones and dihydrochalcones. *Calendula officinalis* also contains various flavonoids which include quercetin, kaempferol, rutin, isorhamnetin, isoquercetin, calendoflaside[30], calendoflavoside and calendoflavobioside, thus having a potential role in antioxidant activity.

Highest flavonoid and flavonol content in GGME and highest total phenolic content in CSME confirm phytochemical screening. Basically, flavonoids are a family of polyphenolics which are synthesized by plants and can be categorised into different

subclasses and each subclass comprises of hundreds of different compounds like anthocyanidins, flavanols, flavones, flavanones, flavonols, flavononols and isoflavones[31]. The activity of flavonoids is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls making them responsible for the radical scavenging effect[32] and chelating process[33] respectively. It has also been reported[34] that phenolic compounds exhibit redox properties by acting as reducing agents, hydrogen donators and singlet oxygen quenchers. Strong relationship[35] was observed between total phenolic content and antioxidant activity in many plant species because phenolic compounds not only attack cell walls and cell membranes by affecting their permeability but also interfere with membrane functions like electron transport, protein synthesis and enzyme activity. Hence, active phenolic compounds can lead to the destruction of pathogens. Furthermore, phenolic compounds directly contribute to the antioxidant action and act as free radical terminators, thereby impairing the inflammatory processes.[36]

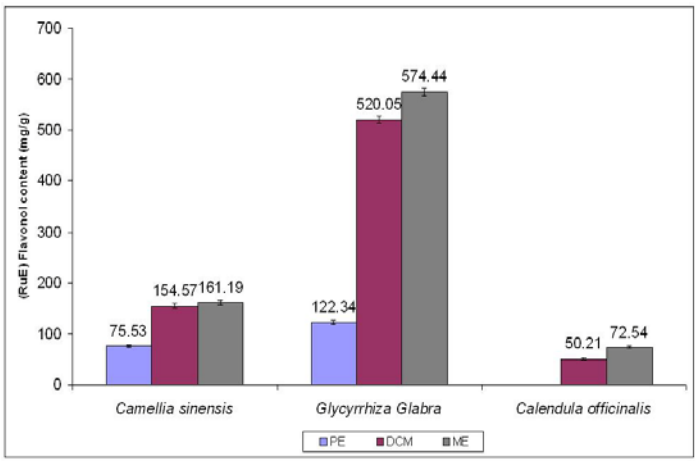


**Fig. 3: Total flavonol content of test extracts**

**Free radical scavenging assays**

In recent years, focus has been on the toxicity related with oxidative stress while treating acne vulgaris because the rate of generation of ROS is more than the rate of its removal. This is due to the continuous production of free radicals even during the normal use of oxygen, as in respiration and other cell mediated immune functions. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induced abnormal proteins [37]. The biological system tries to protect against free radicals with the help of enzymes like SOD and CAT. SOD converts superoxide radicals ($O_2^-$) into water and $O_2$ and similarly if the activity of CAT gets reduced, it leads to the accumulation of superoxide radicals and hydrogen peroxide[38].

**Effect on DPPH radical**

The *in vitro* antioxidant activity of test extracts was estimated using DPPH assay. All methanolic extracts exhibited potent antioxidant activity when DPPH radical was used as a substrate to evaluate the free radical scavenging activity.

The antioxidants reacted with DPPH, a purple color stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The amount of DPPH reduced was estimated by measuring the decrease in absorbance at 517 nm.

Lower $IC_{50}$ value indicated a greater antioxidant activity[39]. Our experimental data indicated that though all the test extracts demonstrated H-donor activity, the highest DPPH radical scavenging activity was observed in CSME ($IC_{50}$ = 44.03 ± 1.784 µg/ml) followed by GGME ($IC_{50}$ = 51.07 ± 3.050 µg/ml) and COME ($IC_{50}$ = 111.96 ± 1.129 µg/ml) presented in (Figure 4).

Mann-Whitney U test showed the comparison of ascorbic acid and active methanolic extracts. P value < 0.05 was observed in COME indicating the data to be significantly different, whereas CSME and GGME showed P value > 0.05.

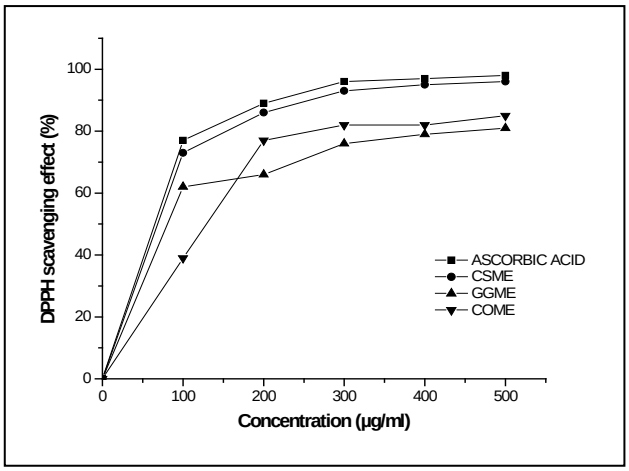


**Fig. 4: DPPH Scavenging effect (%) of methanolic extracts of active medicinal plants and ascorbic acid**

**ABTS radical scavenging activity**

ABTS assay is an excellent tool to determine the antioxidant activity of hydrogen donating and chain breaking antioxidants. $ABTS^{\bullet+}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate. Addition of the plant extracts to this pre-formed radical cation reduced it to ABTS in a concentration-dependent manner. Reduction of free radicals by the test extracts using ABTS was measured at 734 nm. Results showed that GGME, CSME and COME exhibit potent antioxidant activity with $IC_{50}$ values of 21.37 ± 1.422 µg/ml, 28.99 ± 1.544 µg/ml and 33.03 ± 1.784 µg/ml respectively when the results were compared with standard ascorbic acid with an $IC_{50}$ value of 12.86 ± 1.066 µg/ml (Figure 5). P value < 0.05 was observed when ascorbic acid and active methanolic extracts were compared.

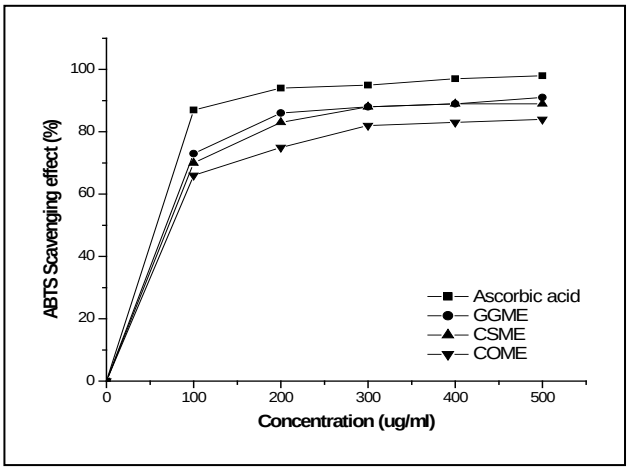


**Fig. 5: ABTS Scavenging effect (%) of methanolic extracts of active medicinal plants and ascorbic acid**

**Metal chelating activity**

Metal chelating capacity of a sample plays an important role as it reduces the concentration of the transition metals which catalyze lipid peroxidation. It has been reported that chelating agents are effective as secondary antioxidants because they decrease the redox potential which ultimately stabilizes the oxidized form of the metal ion[40]. In this assay, Ferrozine forms a violet complex with $Fe^{2+}$ but in the presence of a chelating agent, complex formation gets interrupted which results in a decrease in violet color of the complex. The results clearly demonstrated that the formation of the ferrozine-$Fe^{2+}$ complex is inhibited in the presence of the test and reference compounds. Amongst the thirteen test extracts used for *in vitro* antioxidant activity, CSME, GGME and COME exhibited potent metal chelating activity with $IC_{50}$ values of 234.64 ± 5.467 µg/ml, 331.16 ± 1.972 µg/ml and 458.53 ± 1.393 µg/ml respectively when the results were compared with standard $Na_2EDTA$ with an $IC_{50}$ value of 15.08 ± 1.884 µg/ml (Figure 6). Three methanolic extracts and standard $Na_2EDTA$ showed significant difference ($P<0.05$).

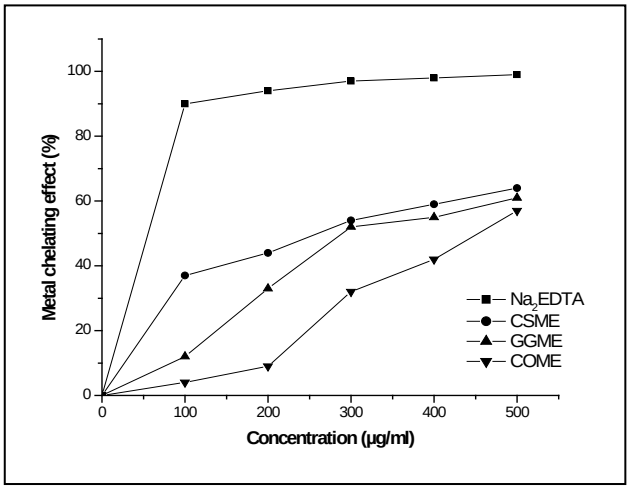


**Fig. 6: Metal Chelating effect (%) of methanolic extracts of active medicinal plants and ascorbic acid**

The chelating ability of the test extracts towards ferrous ions was investigated. Our findings revealed that the plant extracts are not as good as standard $Na_2EDTA$ but the decrease in colour formation in the presence of the extract indicated that they possess iron chelating activity. Ferrous ions are the most effective pro-oxidants and are capable of stimulating lipid peroxidation by the Fenton reaction. They also promote peroxidation process by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals which themselves are capable of abstracting hydrogen and then propagating the chain reaction of lipid peroxidation process.

**CONCLUSION**

The results clearly indicate that methanolic extracts of *C. sinensis*, *G. glabra* and *C. officinalis* possess broad-spectrum antibacterial activity. Presence of higher concentration of phenolic compounds in these test extracts makes them a strong free radical scavenger, which further indicates that these plants can be a good source of natural antioxidants to prevent free radical mediated oxidative stress in acne. Therefore, further investigation is needed to explore the parameters essential for formulation so that antibacterial and

antioxidant potential of these medicinal plants can be utilized to provide safe and effective topical herbal formulation for the treatment of acne.

**REFERENCES**


1. Leyden JJ. Current issues in antimicrobial therapy for the treatment of acne. J Eur Dermatol Venereol 2001; 15 Suppl 3: 51–55.
2. Leyden JJ. Therapy for acne vulgaris. N Engl J Med 1997; 336**:** 1156-60.
3. Goulden V, McGeown CH, Cunliffe WJ. The familial risk of adult acne: a comparison between first degree relatives of affected and unaffected individuals. Br J Dermatol 1999; 141: 297–300.
4. Halliwell B. Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. Am J Med 1991; 91: 14–22.
5. Akamatsu H, Horio T, Hattori K. Increased hydrogen peroxide generation by neutrophils from patients with acne inflammation. Int J Dermatol 2003; 42: 366–369.
6. Stathakis V, Kilkenny M, Marks R. Descriptive epidemiology of acne vulgaris in the community. Australas J Dermatol 1997; 38**:** 115–123.
7. Nithya S, Karthik N, Jayabharathi J. *In vitro* antioxidant activity of hindered piperidone derivatives. Int J Pharm Pharm Sci 2011; 3: 254-256.
8. Porter WL. Recent trends in food applications of antioxidants. In: Autoxidation in Food and Biological Systems. (USA): Plenum Press; 1980.
9. Stapleton PD, Shah S, Anderson JC, Hara Y, Hamilton-Miller JMT, Taylor PW. Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. Int J Antimicrob Agents 2004; 23: 462–467.
12. Toshio Fukai , Ai Marumo, Kiyoshi Kaitou, Toshihisa Kanda, Sumio Terada, Taro Nomura. Antimicrobial activity of licorice flavonoids against methicillin-resistant *Staphylococcus aureus*. Fitoterapia 2002; 73: 536–539.
13. Saeedi M, Morteza-Semnani, Ghoreishi MR. The treatment of atopic dermatitis with licorice gel. J Dermatolog Treat 2003; 14: 153–157.
14. Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Della LR, Tubaro A et al. Anti-oedematous activities of the main triterpendiol esters of marigold (*Calendula officinalis*). J Ethnopharmacol 1997; 57: 139–144.
15. Della LR ,Tubaro S, Sosa S, Becker H, Saar St, Isaac O. The role of triterpenoids in the topical anti-inflammatory activity of *Calendula officinalis* flowers. Planta Med 1994; 60: 516–20.
16. Harborne JB. Phytochemcial methods. (London): Chapman and Hall; 1973.
17. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45:493-496.
18. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem 2001; 73: 73–84.
19. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10: 178–182.
20. Miliauskas, Venskutonis PR,Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004; 85: 231–237.
21. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 26: 1199–1200.
22. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26: 1231–1237.
23. Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 315: 161–169.
24. Kono K, Tatara I, Takeda S, Arakawa K and Hara Y. Antibacterial activity of epigallocatechin gallate against methicillin-resistant *Staphylococcus aureus*. Kansenshogaku Zasshi 1994; 68: 1518-1522.
25. Capasso F, Mascolo N, Autore G, Duraccio MR. Glycyrrhetinic acid, leucocytes and prostaglandins. J Pharm Pharmacol 1983; 35: 332-335.
26. Young HL, In-Hwan K, Jung-Ju S. *In vitro* activity of kaempferol isolated from the *Impatiens balsamina* alone and in combination with erythromycin or clindamycin against *Propionibacterium acne.* J Microbiol 2007; 10: 473-477.
27. Yen GC, Duh PD, Tsai CL, Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem 1993; 41: 67–70.
28. Mitscher L, Jung M, Shankel D, Dou J, Steele L, Pillai S. Chemoprotection: A review of the potential therapeutic antioxidant properties of green tea (*Camellia sinensis*) and certain of its constituents. Med Res Rev 1997; 17: 327–365.
29. Fujiki H, Yoshizawa S, Horiuchi T, Suganuma M, Yatsunami J, Nishiwaki S et al. Anticarcinogenic effects of (-)-epigallocatechin gallate. Prev Med 1992; 21: 503–509.
30. Toshio F, Kazue S, Taro N. Preliminary evaluation of anti-nephritis and radical scavenging activities of glabridin from *Glycyrrhiza glabra* Linn. Fitoterapia 2003; 74: 624–29.
31. Guoxiu X, Nan L, Tong W, Meiyan Y. Advances in studies on flavonoids of Licorice. Zhongguo Zhong Yao Za Zhi 2003; 28:593–597.
32. Vidal-Ollivier E. Flavonol glycosides from *Calendula officinalis* flowers. Planta Med 1989; 55: 73–73.
33. Beecher GR. Overview of Dietary Flavonoids: Nomenclature, Occurrence and Intake. J Nutr 2003; 133**:** 3248–3254.
34. Younes M. Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. Planta Med 1981; 43: 240–245.
35. Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol 2003; 55: 131–142.
36. Rice Evans CA, Miller NT, Paganga G. Antioxidant properties of selected fruit seeds. Food Chem 1997; 88: 411–417.
37. Oktay M, Culcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foenniculum vulgare)* seed extracts. Lebensm Wiss Technol 2003; 36: 263–271.
38. Shahidi F, Wanasunadra PKJ. Phenolic antioxidants. Crit Rev Food Sci Nutr 1992; 32: 67–103.
39. Torane RC, Kamble GS, Khatiwora E, Ghayal NA, Deshpande NR.Antioxidant capacity of leaves and stem of *ehretia laevis*. Int J Pharm Pharm Sci 2011; 3: 149-151.
40. Sampathkumar R, Siva Kumar T, Siva Kumar P, Netaji R, Vijayabaskar M, Perumal P et al. Hepatoprotective and *in vivo* antioxidant effects of *Careya arborea* against carbon tetrachloride induced liver damage in rats. Int J Mol Med and Adv Sci 2005; 1**:** 418–424.
41. Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. Biosci Biotechnol Biochem 1998; 62: 1201–1204.
42. Gardner PT, White TC, McPhail DB, Duthie GG. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chem 2000; 68: 471- 474.