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COCCULUS LAURIFOLIUS: A RICH ANTIMICROBIAL, ANTIOXIDANT AND PHYTOCHEMICAL SOURCE

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Abstract

The study was carried out to investigate the antimicrobial, antioxidant potential and the qualitative and quantitative phytochemical analysis of the bark and leaf of *Cocculus laurifolius* DC. by using polar and non-polar solvents, i.e. Petroleum ether, Chloroform, Methanol and distilled water. Chloroform bark extracts showed maximum % yield. Antimicrobial activity was determined by using 4 bacterial strains (2 gram-negative and 2 gram- positive) and 2 fungal strains. Leaf and bark extracts of *C. laurifolius* showed significant to average results against bacterial and fungal strain. Bark extracts of chloroform and methanol revealed a maximum zone of inhibition against *S. aureus* in agar-well diffusion method with values of 37±3.1mm and 37±2.2mm respectively and bark extract of methanol exhibited MIC value with 0.06±0.01 (at 0.9 mg/L) against *E. coli.* In antifungal activity, all extracts showed average results against fungal strains. Maximum result exhibited by bark extract of methanol with values 29±1.4 and 0.70±0.01 (at 1 mg/L) against *F. solani* in zone of inhibition and MIC analysis. Significant DPPH free radical scavenging activity of chloroform extracts of bark i.e. 92.45±0.1 while extract of chloroform bark display IC_{50}: 28.27±1.03 µg/mL. Antioxidant activity of plant further confirmed by conducting three more activities i.e. Total phenolic contents (TPC), Metal chelating (MC) and Total antioxidant activity (TAA). Chloroform extracts of bark showed a maximum value of TPC, i.e. 293±1.3 GAE mg/mL while bark extract of methanol exhibited maximum MC value, i.e. 262±0.01mg/mL and leaf extracts of methanol showed highest TAA value, i.e. leaf 1.1753± 0.3. *Cocculus laurifolius* contain many phytochemical constituents, i.e. Alkaloids, flavonoids, saponins, tannins and phenolic compounds Bark contain high amount of alkaloids and flavonoids, i.e., 17.13±0.78 and 16.73±0.56 respectively as compared to leaf but phenolic contents and saponins were present in leaf in significant amount.

Key words: *Cocculus laurifolius,* Antimicrobial, Antioxidant, Phytochemicals

Introduction

Plants have importance in our lives more than animals due to their extraordinary array of different classes of biochemicals also with a variety of biological activities (Cotton, 1996). In last few years it was analyzed that about 80% of population in developing countries depend on herbal medicines for their health care (Kirby. 1996). Many higher plants mount up extractable organic approaches. Plants are a rich source of medicines which are used against microbes because they manufacture large collection of bioactive molecules, most of which probably evolved as chemical defense against infection. Plants have also been considered as rich source of antibiotics due to their higher antimicrobial potential against microbes. It is expected that only 1% out of 2, 65,000 flowering plants on earth have been studied thoroughly for their chemical composition and potential against microorganisms (Cox & Balick, 1994). Oxidation processes are very important in all organisms and different disorders namely cancer, coronary heart disorders and diabetes diseases caused due to mechanism of uncontrolled production of oxygen free radicals and antioxidant defense (Erlund *et al*., 2001; Yam *et al*., 2008). Plants are considered being exhibiting the best antioxidant potential due to the presence of phenolic contents because antioxidant properties of plant extracts have been documented to their polyphenolic content (Lu & Foo. 2001; Murthy *et al*., 2002).

Plants contain many phytochemical contents namely isocatechins, catechins, (polyphenolic constituents), anthocyanin, alkaloids, flavonoids, isoflavones, sapponins, tannins, phenols, lignins and coumarines due to these compound plant exhibit strong antimicrobial and antioxidant activities (Aqil *et al*., 2006).

C. laurifolius (Fig. 1) belongs to family Menispermaceae and also known as laurel-leaved snail tree. From 35 species of genus *Cocculus* DC. only two have been reported in Pakistan by Siddiqi (1974). *Cocculus laurifolius* DC. is shrub or considered as small tree upto 1-2m high with striate, glabrous branches and branchlets (Ajaib & Khan, 2012). It is spreading in Shrublands and open forests of N.W. Hunan, Taiwan, Xizang (Gyirong), Indonesia, Japan, Laos, Malaysia, Myanmar, Thailand, Nepal, India (Tamil Nadu) & South East Asia (Ji, 2008; Kottaimuthu *et al.*, 2008).

Material and Methods

Plant material: Plant *cocculus laurifolious* was collected from Botanic Garden, GC University, Lahore in the month of July 2013, and was identified and deposited DR. Sultan Ahmed herbarium Department of Botany, GCU Lahore with voucher specimen NO. SAH. 2364 for further reference.

Maceration of plant material: Leaf and bark of the plant dried under shade and macerated in different solvents according to their polarity index (Petroleum ether, Chloroform, Methanol, Aqueous). The resultant extracts were dried on rotary evaporator to get final concentrated form of extracts. After the determination of % yield of extracts, that were further used to investigate the antimicrobial and antioxidant activity and for the qualitative and quantitative analysis.

Fig. 1. *Cocculus laurifolius* DC. in natural habitat.

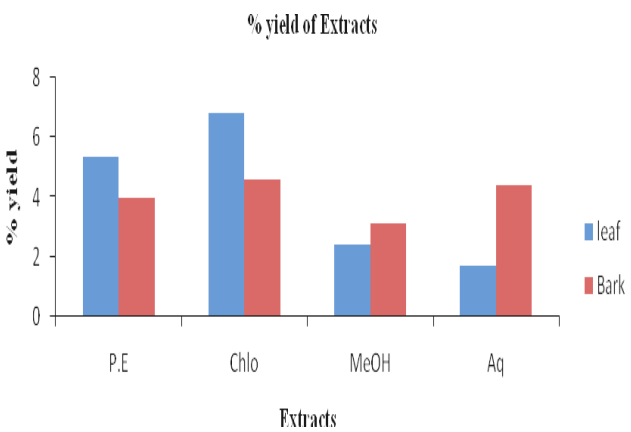

Fig. 2. % extraction yield of leaf and bark of *C. laurifoloius* DC.

Antimicrobial activity

Zone of inhibition assay: Investigation of antimicrobial activity was carried out by using Agar well diffusion method (Bauer *et al*., 1996). Autoclaved prepared medium (Nutrient-agar following Cruick-shank *et al*., 1975 and Potato Dextrose Agar in accordance to Johansen (1940) were used for investigation of antibacterial and antifungal activity respectively.

Evaluation of minimum inhibitory concentration (MIC): Plant samples having antimicrobial potential with lower constancy evaluated by using Broth-dilution method followed by Murray *et al*. (1999).

Antioxidant assays

DPPH free radical scavenging activity of *C. laurifolius*: The activity was carried out using method of Lee & Shibamoto (2001). In brief, 1ml of (500, 250, 125, 60µg/mL) of plant extract of respective plant, i.e., *C. laurifolius* was taken followed by 3ml of 0.1mM DPPH, shaken vigorously and incubated at room temperature for 1 hour. Then absorbance was taken at 517 nm against methanol as a blank in the spectrophotometer. Lower absorbance indicates higher free radical scavenging activity. The % DPPH discoloration of the samples was calculated by using following formula:

$$\text{Antiradical activity} = \frac{\text{A (control)} - \text{A (sample)}}{\text{A (control)}} \times 100$$

IC_{50} values of the plant samples were identified for the evaluation of radical scavenging activity of the plant samples using Microsoft Excel.

Total antioxidant activity by phosphomolybdenum method (TAA): Total antioxidant activity of respective plant was investigated by phosphomolybdenum complex formation method (Prieto *et al*., 1999). In test tubes 500µg/mL of each extract was taken followed by 4mL of reagent solution (0.6M H_2SO_4, 28mM Na_2HPO_4 and 4mM $(NH_4)_6Mo_7O_{24}.4H_2O$). The blank solution contained only 4 mL of reagent solution. Test tubes containing reagent and plant extract were capped and allowed to incubate for 90 minutes in water bath at 95ºC. Samples were cooled at room temperature after the accomplishment of incubation time and the absorbance of solution was taken at 695 nm against blank and the antioxidant activity was expressed comparative to BHT.

Total pehnolic contents (TPC): Total phenolic contents of *C. laurifolius* were determined by using the methodology applied by Makkar *et al*. (1993). 100µg/mL of the respective plant extracts with the consistency of 0.5mg/mL was taken in test tubes followed by 2.8mL of 10% Na_2CO_3 and 0.1mL of 2N FC reagent.

Metal chelating assay (MC): The chelation of ferrous ion by extracts was estimated using method of Dinis *et al*., (1994). Briefly 50µL of 2mM $FeCl_2$ was taken in test tubes followed by 1mL of plant extract, 32mg/mL and 0.2mL of 5mM ferozine solution. The mixture was shaken vigorously and allowed to stand at room temperature for 10 minutes. The absorbance was taken at 562nm. The percentage inhibition of ferozine- Fe^{+2} complex formations was calculating by using underneath formula:

$$\% \text{ Inhibition} = A^0 - As/As$$

where, A^0 is absorbance of control and A_s is absorbance of respective sample.

Qualitative analysis of phytochemicals: The qualitative analysis of phytochemicals constituents was done by using methodology of (Harborne, 1973).

Quantitative analysis of phytochemicals: The quantitative analysis was carried out by employing the methodology of (Harborne, 1973; Pearson, 1976).

Results and Discussion

The % yield of plant extracts was evaluated (Fig. 2) to know about the commercial and economical aspect of plant. Bark extract of chloroform exhibited maximum % yield (4.6%) followed by P.E extract (4.4%), aqueous extract (4%) and Methanol extract (3.12%). In comparison to bark, leaf extracts had a minimum % yield. Leaf extracts of chloroform showed significant % yield (4.51%), proceeded by petroleum ether extracts (4.16%), methanol (2.4%) and aqueous extract (1.72%).

For the investigation of antimicrobial activity of bark and leaf of this plant, 2 gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), 2 gram positive *(Bacillus subtilis* & *Staphylococcus aureus*), and 2 fungal strain *Aspergillus niger* & *Fusarium solani*) were used. The results were compiled in comparison with slandered disc for antimicrobial significance (Tables 1 & 2). To provide evidence that the antimicrobial potential was exhibited purely by plant specimen and solvents were not responsible, respective solvents, i.e. petroleum ether, chloroform; methanol and distilled water were used as negative control (Tables 3 & 4).

Both plants parts showed good results against bacterial strains (Table 5) (Figs. 3, 4, 5, 6 & 7), bark extracts of chloroform and methanolic extracts displayed excellent results, i.e. 37±3.1 and 37±2.1 against *S. aureus* respectively as compared to standard disc Azithromycin with a value, i.e. 13±0.7, while leaf extracts of methanol, chloroform and P.E showed significant zone of inhibition against *E. coli* with value, i.e. 37±0.5, 34±1.5 and34±2.3 mm respectively as compared to standard disc, Cephradin with a value, i.e., 24±1.5, all extracts showed zone of inhibition against all bacterial strains. Similar results were also observed by (Abraham & Thomas, 2012) while working on *Cyclea paltata* by using gram-negative and gram-positive strains. The antimicrobial feature demonstrated by both bark and leaf of *C. laurifolius* followed same trend: Methanol >Petroleum ether> Chloroform > Aqueous in respect to solvents Abed *et al*., (2013) also found similar results while working on *Gynotroches axillaris* Blume with different solvent extracts like petroleum ether, chloroform and methanol. Methanol extract had higher contents of hydrophilic phenolic compounds which become active against gram-negative bacteria.

Table 1. Zone of inhibition produced by standard antibacterial disc (Positive control)

Antibacterial standard disc	Conc. (µg)	Bacterial strains	Zone of inhibition (mm)
Amikacin	30	*B. subtilis*	18 ± 0.5
Azithromycin	15	*S. aureus*	13 ± 0.7
Cephradine	30	*E. coli*	24 ± 1.5
Ampicillin	10	*P. aeruginosa*	23 ± 0.6

Table 2. Zone of inhibition produced by standard antifungal disc (Positive control).

Antifungal standard disc	Conc.(µg)	Fungal strains	Zone of inhibition (mm)
Voriconazole	100	*A. niger*	40 ± 2.0
Itraconale	100	*F. solani*	10 ± 1.8

Table 3. Zone of inhibition of solvents against bacterial strains (Negative control).

Solvent	Quantity	Zone of inhibition against Bacterial strains (mm)			
		S. aureus	*E. coli*	*P. aeruginosa*	*B. subtilis*
Petroleumether	1.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Chloroform	1.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Methanol	1.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Aqueous	1.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Final response		**Negligible**	**Negligible**	**Negligible**	**Negligible**

Table 4. Zone of inhibition of solvents against fungal strains (Negative control)

Solvent	Quantity (ml)	Zone of inhibition against fungal strains (mm)	
		A. niger	*F. solani*
Petroleum ether	1.5	0 ± 0	0 ± 0
Chloroform	1.5	0 ± 0	0 ± 0
Methanol	1.5	0 ± 0	0 ± 0
Aqueous	1.5	0 ± 0	0 ± 0
Final response		**Negligible**	**Negligible**

Table 5. Zone of Inhibition produced by bark & leaf of *C. laurifoloius* DC. against bacterial strains.

Plant part	Extracts	Zone of inhibition (mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
Leaf	Petroleum ether	34 ± 2.3	30 ± 1.1	21 ± 3.0	25 ± 3.1
	Chloroform	34 ± 1.5	20 ± 2.1	29 ± 1.1	27 ± 1.4
	Methanol	37 ± 0.5	24 ± 1.7	22 ± 1.8	23 ± 2.4
	Aqueous	25 ± 0.1	21 ± 1.2	26 ± 2.7	25 ± 2.5
Bark	Petroleum ether	36 ± 4.0	35 ± 2.1	29 ± 2.9	36 ± 3.2
	Chloroform	31 ± 3.2	37 ± 3.1	33 ± 2.7	33 ± 2.4
	Methanol	34 ± 2.0	37 ± 2.2	31 ± 2.0	26 ± 0.8
	Aqueous	22 ± 1.7	22 ± 1.4	27 ± 1.2	28 ± 1.1

Fig. 3. Zone of inhibition produced by: Bark extract against (a). *E. coli* (Chloroform), (b). *B. subtilis* (P.E), (c). *P. aeruginosa* (P.E), (d). *P. aeruginosa* (Chloroform), (e). *E. coli* (Methanol), (f). *E. coli* (Aqueous), (g). *B. subtilis* (Aqueous), (h). *F. solani* (Chloroform.); Leaf extracts against (i). *F. solani* (Methanol).

The MIC of methanolic extracts of *C. laurifolius* had exhibited significant resistance against gram-negative and gram-positive bacteria. The most significant results of methanolic extracts were obtained against *E. coli* by bark, i.e. 0.069±0.01 and 0.07±0.02 against *P. aeruginosa* but *B. subtilis* showed susceptibility against both bark and leaf extracts, i.e., 0.14±0.02 and 0.28±0.01 respectively (Table. 6).

Both leaf and bark extracts showed average to poor results against fungal strains (Table 7); (Figs. 3 & 8). *A.niger* is highly susceptible against leaf and bark extracts. All extracts produced zone of inhibition against *F. solani* except petroleum ether because *F. solani* showed high susceptibility against petroleum ether extracts. Maximum zone of inhibition is produced by methanol and chloroform extracts with a value 29±1.4 and 29±08 respectively as compared to standard disc Atraconozol with a value, i.e., 10±1.8. The antifungal activity demonstrated by both bark and leaf extracts follow same trend Methanol > Chloroform > Aqueous>Petroleum ether in respect to solvent and *A. nigar* > *F. solani* for resistance against test-strain as well. It is demonstrated that both leaf and bark extracts are highly active against bacterial strain. All extract showed satisfactory results against *F. solani* as compared to *A. nigar*. Same results were listed by Gul *et al*. (2012) during the study of antimicrobial activity of different extracts of leaf and branches of *Datura stramonium*.

The MIC of methanolic extracts of *C. laurifolius* had exhibited significant resistance against fungal strains (Table 8). The extracts showed good results against *F. solani*. Maximum resistance was offered by Bark (0.70±0.01) and leaf (0.309±0.01) while *A.niger* showed high resistance against both bark and leaf.

Antioxidant potential was investigated by using 4 tests, i.e. DPPH, Total phenolic contents, Metal chelating assay and Total antioxidant assay. In DPPH assay various concentrations were required while in investigation of antioxidant potential by TAA, MC and TPC only 500µg/mL concentration was used.

The % free radical scavenging activity of the sample was investigated by using DPPH solution and different concentration is employed (Figs. 9, 10). Bark extract of chloroform showed a good result (93.53±0.6% at 250 µg/mL) proceeded by leaf extracts of methanol (90.21±2.6 at 250 µg/mL) while less capability showed by Aqueous extracts of bark (24.01±0.8 µg/mL). The radical scavenging property of plant ranges from Good to satisfactory. Same results were listed by (Ebrahimzadeh *et al*., 2008a) while working on antioxidant activities of Iranian Corn Silk. The values of % inhibition were plotted against their respective inhibition to provide deterioration curve, useful in the determination of IC_{50}. The results obtained in (Table 9) revealed that the bark extracts of chloroform displayed (28.27±1.02µg/mL). The similar results noted by Ajaib *et al*., (2013a) during the estimation of antioxidant and antimicrobial activities of *Holmskioldia sanguinea* Retz.

The total phenolic contents were determined in comparison to the Galic acid (Table 10) (Fig. 11). Maximum activity was exhibited by bark extracts of Chloroform with a value, i.e. 293±1.9 GAEmg/mL followed by aqueous extract of bark (265.07±1.7 GAEmg/mL) and Methanolic extract of Leaf (155±1.2 GAEmg/mL). However, other extracts have less pehnolic content lowest value demonstrated by petroleum ether extracts of leaf (59±0.5) same results listed by (Ajaib *et al*., 2013b) while working on *Rivina humilis* L: A potential antimicrobial and antioxidant source.

Metal chelating activity was employed for the determination of chelation of F^{+2} ions (Table 11) (Fig. 12). Both methanolic extracts of bark (262±0.01) and leaf (251±0.02) displayed a maximum metal chelating activity and both aqueous extracts bark (129±0.0005) and leaf (115±0.006) showed lowest metal chelating activity. Ebrahimzadeh *et al*., (2008b) listed same results while working on Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran.

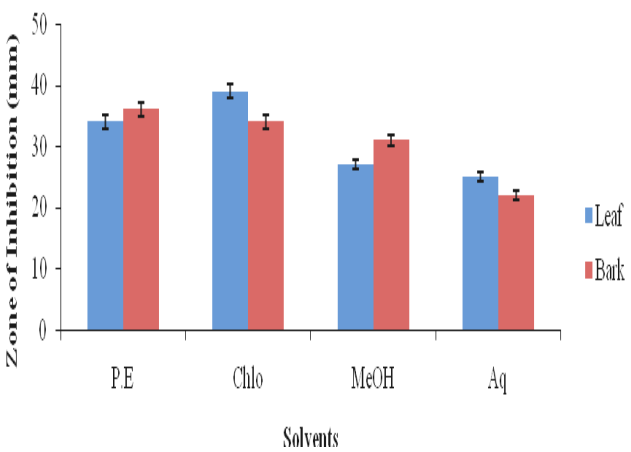

Fig. 4. Zone of inhibition of leaf and bark extracts of *C. laurifolius* against *E. coli*.

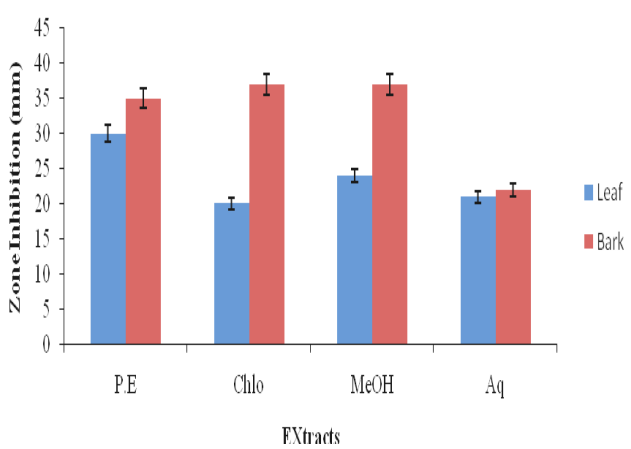

Fig. 5. Zone of inhibition of leaf and bark extracts of *C. laurifolius* against *S. aureus*.

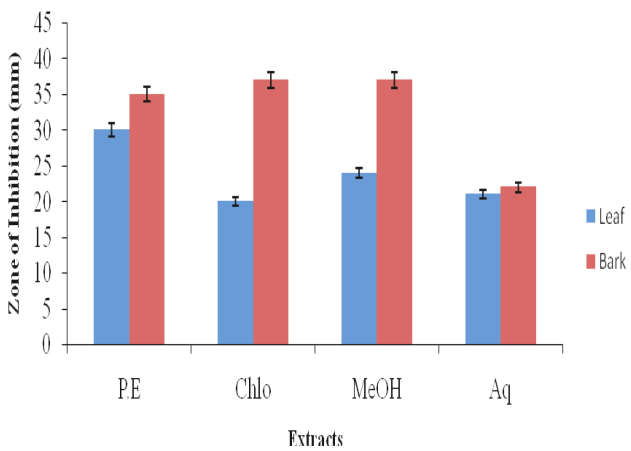

Fig. 6. Zone of inhibition of leaf and bark extracts of *C. laurifolius* against *P. aeruginosa*.

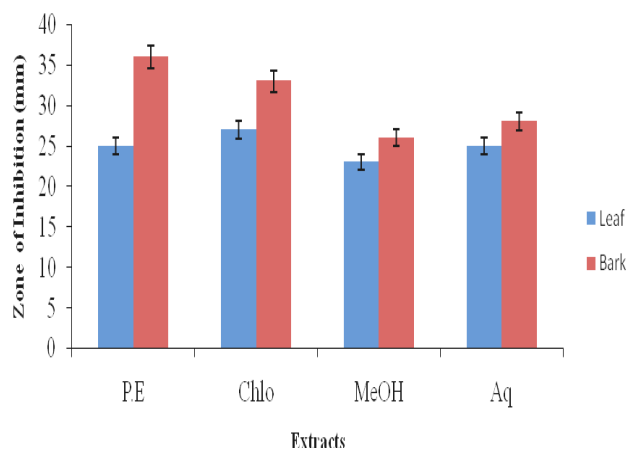

Fig. 7. Zone of inhibition of leaf and bark extracts of *C. laurifolius* against *B. subtilis*.

Table 6. MIC values of Bark & Leaf extracts of *C. laurifoloius* DC. against bacterial strains

Plant part	Bacteria							
	E. coli		*P. aregenosa*		*B. subtilis*		*S. areus*	
	Conc.	MIC	Conc.	MIC	Conc.	MIC	Conc.	MIC
Leaf	1	0.17 ± 0.02	0.8	0.15 ± 0.03	0.4	0.28 ± 0.01	0.6	0.05 ± 0.04
Bark	0.9	0.06 ± 0.01	0.8	0.07 ± 0.02	0.6	0.14 ± 0.02	0.8	0.18 ± 0.02

Table 7. Zone of Inhibition produced by bark & leaf of *C. laurifoloius* DC. against fungal strains.

Plant part	Solvent	Zone of inhibition	
		Fusarium	*Aspergilus niger*
Leaf	Petroleum Ether	0 ± 0	0 ± 0
	Chloroform	26 ± 1.4	0 ± 0
	Methanol	27 ± 0.5	0 ± 0
	Aqueous	25 ± 0.5	0 ± 0
Bark	Petroleum Ether	0 ± 0	0 ± 0
	Chloroform	28 ± 0.8	12 ± 1.5
	Methanol	29 ± 1.4	0 ± 0
	Aqueous	23 ± 0.9	0 ± 0

Table 8. MIC values of Bark & Leaf extracts of *C. laurifoloius* DC. against fungal strains

Plant part	Fungus			
	Fusarium		*Aspergillus niger*	
	Conc.	MIC	Conc.	MIC
Leaf	0.9	0.309 ± 0.01	0.8	0.288 ± 0.03
Bark	1	0.70 ± 0.01	0.7	0.177 ± 0.02

Table 9. IC_{50} value of bark and leaf extracts of *C. laurifoloius* DC. using DPPH.

Plant part	Solvents	*IC* $_{50}$ value
Leaf	Petroleum ether	54.59 ± 0.03
	Chloroform	84.930 ± 1.2
	Methanol	37.250 ± 0.09
	Aqueous	104 ± 1.5
Bark	Petroleum ether	78.0 ± 0.5
	Chloroform	28.27 ± 1.02
	Methanol	71.66 ± 1.12
	Aqueous	125 ± 0.8

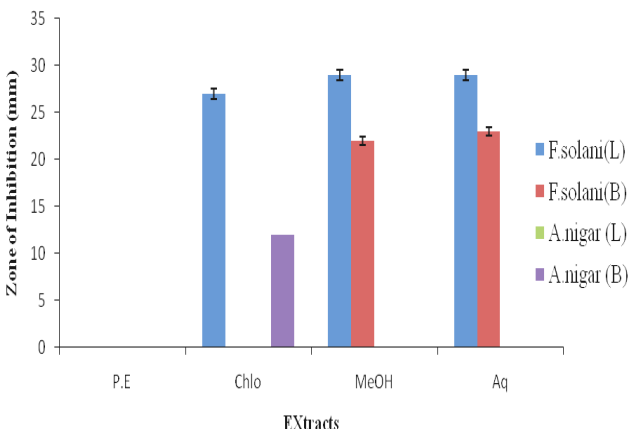

Fig. 8. Zone of inhibition of leaf and bark extracts of *C. laurifolius* against *F. solani*.

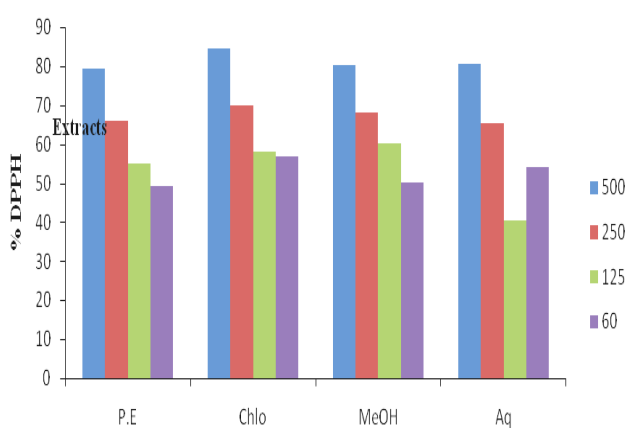

Fig. 9. % Free radical scavenging activity of *C. laurifolius* DC. leaf extracts using DPPH.

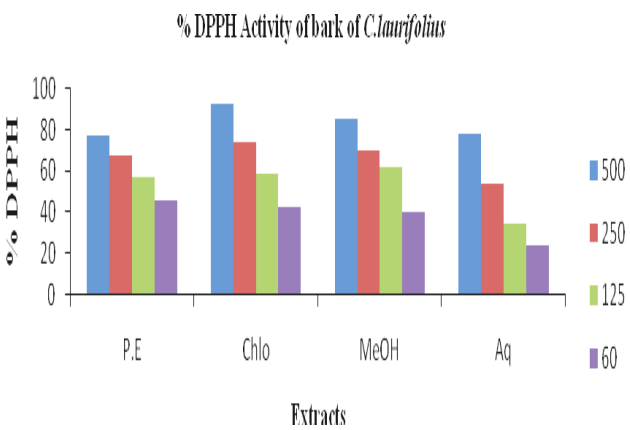

Fig. 10. Free radical (%) scavenging activity of *C. laurifolius* DC. bark extracts using DPPH.

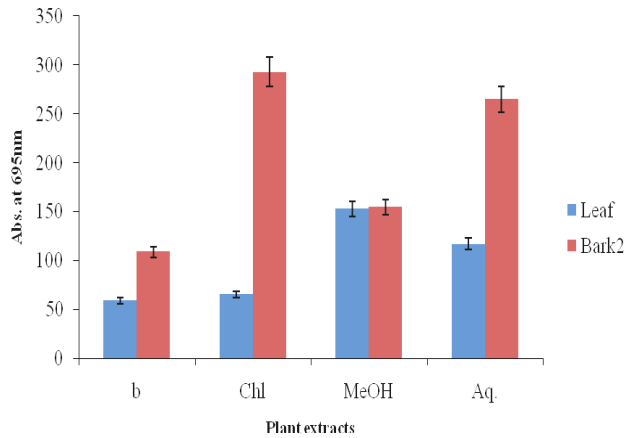

Fig. 11. Phenolic concentration of *C. laurifolius* bark and leaf extracts using TPC method.

Table 10. Total phenolic contents of *C. laurifolius*.

Plant part	Extracts	Absorbance	Amount of TPC
Leaf	Petroleum ether	0.493 ± 0.5	59
	Chloroform	0.432 ± 0.8	65.5
	Methanol	1.061 ± 1.2	153
	Water	0.845 ± 3.7	117
Bark	Petroleum ether	0.793 ± 3.4	109
	Chloroform	1.752 ± 1.9	293
	Methanol	1.732 ± 2.3	265
	Water	1.07 ± 1.7	155

Table 11. Metal chelating assay of *C laurifolius*.

Plant part	Solvent	Absorbance	% Inhibition
Leaf	P.E	0.40 ± 0.005	160
	Chlo	0.44 ± 0.008	145
	MethOH	0.33 ± 0.02	251
	Water	0.51 ± 0.0006	115
Bark	P.E	0.390 ± 0.003	144
	Chlo	0.15 ± 0.04	159
	MethOH	0.32 ± 0.01	262
	Water	0.53 ± 0.005	129

Table 12. Total antioxidant activity of *C .laurifolius*.

Plant part	Extracts	Absorbance
Leaf	Petroleum ether	1.034 ± 0.0
	Chloroform	1.048 ± 0.1
	Methanol	1.173 ± 0.3
	Water	1.105 ± 0.1
Bark	Petroleum ether	1.1036 ± 0.0
	Chloroform	1.105 ± 0.1
	Methanol	1.144 ± 0.0
	Water	1.757 ± 0.4
Standard		1.2 ± 0.1

Total antioxidant activity was examined by using phosphomolybednum method (Table 12; Fig. 13). The maximum total antioxidant activity was showed by leaf extracts of methanol, i.e., 1.175± 0.3 and bark, i.e., 1.1484±0.0 close immediacy to BHT standard with a value 1.218±0.07. The trend was observed in extracts regards to antioxidant activity Methanol > Chloroform > Petroleum ether > Aqueous. According to this trend aqueous showed minimum antioxidant activity. Riaz *et al*., (2012) reported similar results while investigating oxidative potential of *Cotinus coggyria*.

Qualitative and quantitative analysis of phytochemicals was also carried out (Table 13) (Fig. 14). Bark contained phenolic compounds (28.33±0.88) followed by alkaloids (17.13±0.78) flavonoids (16.73±0.56), saponins (13.66±0.88) and tannins (11.68±0.67) while leaf also contained high amount of phenol (25.33±0.08) proceeded by saponins (20.4±0.43), alkaloids (13.53±0.05), flavonoids (12.16±0.95) and tannins (6.04±0.57). Leaf extracts had less amounts of tannins (Fig. 14) as compared to bark similar observations were also documented by Aqil *et al*., (2006) while working on Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants.

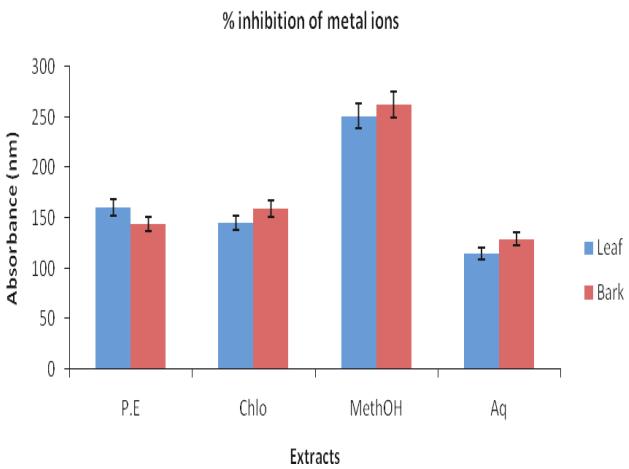

Fig. 12. Metal chelating assay of *C. laurifolius* DC.

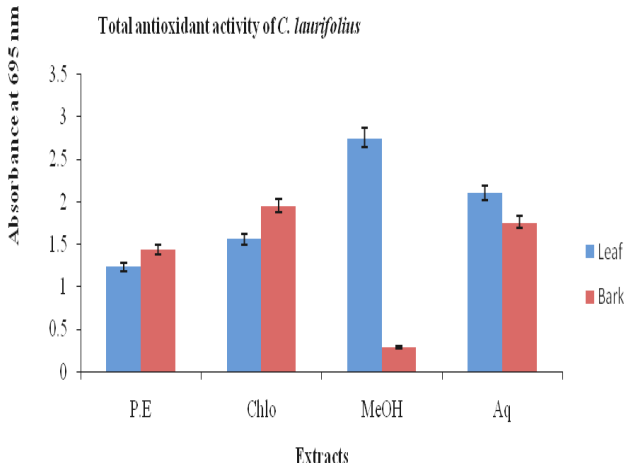

Fig. 13. Total Antioxidant activity of bark & leaf of *C. laurifolius* DC.

Table 13. Quantitative analysis of phytochemical compounds.

Plant part	Amount of phytochemical contents (%).				
	Alkaloids	Flavonoids	Phenol	Sapponins	Tannis
Leaf	13.53 ± 0.05	12.16 ± 0.95	35.33 ± 0.08	20.4 ± 0.43	6.04 ± 0.57
Bark	17.13 ± 0.78	16.73 ± 0.56	48.33 ± 0.88	13.66 ± 0.88	11.68 ± 0.67

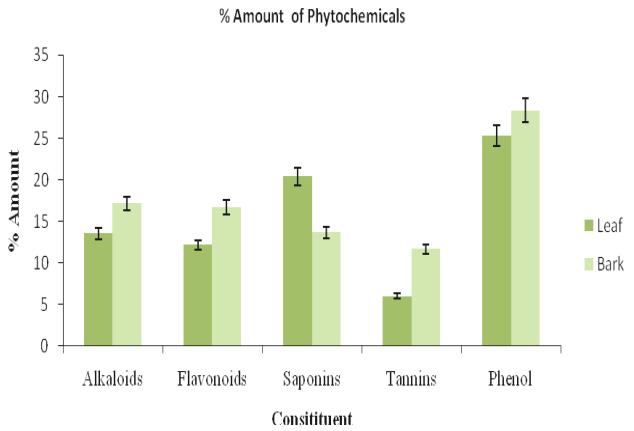

Fig. 14. Amount of Phytochemicals (%) present in bark and leaf of *C.laurifolius.*

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