Research Article

Phytochemical, antibacterial and antifungal activities of leaves and bark of *Colebrookea oppositifolia*: an ethnomedicinal plant

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Abstract

The phytochemical, antibacterial, antifungal and MIC (minimum inhibitory concentration) study of the leaves and bark of *Colebrookea oppositifolia* Smith was evaluated. Phytochemical screening *C. oppositifolia* Sm. indicated the presence of alkaloids, flavonoids, glycosides, steroids and saponins whereas terpenoids, tannins, and cardiac glycosides are showed only in bark extract. Petroleum ether extract showed maximum effectiveness against *P. aeruginosa* with bark extract (18 ± 2.2 mm) and maximum antifungal potential exhibited by petroleum ether extract of leaves (15 ± 0.3 mm) against *A. nigar*. It was noticed that the extracts in distilled water had not shown much potential. The MIC assay was carried out for further verification of results obtained by the Zone of Inhibition against both bacterial and fungal strains. The lowest MIC value is 0.09 ± 0.3 at 1 mg/mL against *E. coli* exhibited by bark extract and 0.09 ± 0.2 at 0.9 mg/mL against *K. pneumoniae* shown by leaf extract. Whereas, in fungi minimal MIC value exhibited 0.11 ± 0.3 at 0.9mg/mL by leaf extract against *A. oryzae*. Hence, it is recommended further investigation to explore toxicological and clinical trials before using this plant as drug.

Keywords: Antibacterial; Antifungal; *Colebrookea oppositifolia*; Ethnomedicinal plant

Introduction

Products obtained from plants have been used medicinal purposes since pre-historic time. For instance, certain conventional pharmaceuticals including numerous trees, bushes, climbers and herbs have been utilized to remedy of certain diseases. Traditionally important plants have become sources for examining their bioactive

properties by the identification of a critical number of helpful compounds [1, 2].

Ethno-botanical plants frequently use as pharmaceuticals, Nutraceuticals, makeup and nourishment supplements [3]. With an expansion of resistant microorganisms, conventional plants are being researched for their antibacterial and therapeutic qualities. The antibacterial and antifungal impact of a plant is because of the

phytochemicals such as saponins, phenolic compounds, tannins, terpenoids, heart glycosides, alkaloids and flavonoids [4]. Numerous emphasis made on deciding the antimicrobial action of plant concentrates found in local societies in the form of oils, flavonoids, alkaloids, lactones etc.[5].

Foodborne diseases fall out 76 million individuals every year in the United States, represent almost 325,000 hospitalizations and more than 5,000 passings. The diseases caused by food are generally dangerous, even fatal for example, microscopic organisms, growths, infections and parasites that enter the body essentially by the ingestion of debased sustenance [6]. Because of the rapid resistance to antibiotics by using allopathic medicines, there is a need to grow new and inventive antimicrobial compounds. As plants are cheap, non-poisonous source, there is a long convention of utilizing them as a part of the treatment of irresistible illness in Cameroonian people[7]. There are numerous strategies for hindering or inactivating microorganisms by utilizing plant derived products as antimicrobials [8].

Colebrookea oppositifolia Smith (Figure1) is a genus of family Lamiaceae/Labiatae. *C. oppositifolia* is commonly known as Bansa. It is medicinally important plant and it is utilized locally as sickness for different purposes. The leaves paste is utilized for wound healing and bruises while the paste of its roots is utilized against the epilepsy [9]. Juice assimilated by beating young leaves is utilized as anthelmintic [10]. The root and stem juice are used in eye diseases. It has intriguing antibacterial action and had exceptional radical searching potential to be utilized as cell reinforcement [11]. Keeping in view the significance of ethno botanical status of *C. oppositifolia* Smith and other members of family Lamiaceae the present investigation was carried out.

Materials and methods

Plant material

The plant *Colebrookea oppositifolia* Sm. commonly known as Bansa Siyah was collected from the Kakra town, District Mirpur AJK. Some plants of that species also seen in Khoirata, Peer Gali and Kotli. This plant belongs to the family Lamiaceae, also called as Mint family. The plant species collected was mounted on herbarium sheet and submitted in the Herbarium Department of Botany MUST, Bhimber campus. A voucher No. MUST.

BOT. 880 to obtain for further reference.

Figure 1. *Colebrookea oppositifolia*

Test organisms

Total six bacteria were selected, the three strains were from gram positive *Enterococcus faecalis*, *Staphylococcus aureus* and *Bacillus subtilis* and two strains of gram negative *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* and two strains of fungal i.e. *Aspergillus oryzae* and *Aspergillus niger* were selected used for investigation of antimicrobial perspective of *C. oppositifolia* Smith. Four standard antibiotics of bacterial strains (azithromycin, methicillin, amoxicillin and ampicillin) and four standard antibiotics of fungal strains (nystatin, tezole, fungivin, and griseofulvin) were tested for comparing the potential of plant extract with them.

Methodology adopted

The whole plant was washed with cold water for removing of mud. After washing the plant parts such as bark and leaves were

separated and kept for drying under room temperature about 20-30 days. After drying the powder was made with the help of pestle and mortar and then exposed for maceration method [12].

Maceration of the plant specimen

The shade dried plant material of *C. oppositifolia* was subjected for maceration different solvents i.e. Petroleum ether,

Chloroform, Methanol and distilled water and then evaporated to get final concentrated form of extracts. The corporeal analysis of herbal extracts considered its texture, colour and appearance, and calculated yield before further study was accompanied.

Yields percentage (%) was calculated with the following method:

$$\text{Extraction yield} = \frac{\text{Weight of plant extract}}{\text{Weight of initial plant sample}} \times 100$$

Qualitative study of phytoconstituents

The phytochemical investigation of plant extracts were done by using typical process [13].

Estimation of antimicrobial activity

By using agar well diffusion method for the antimicrobial activity of leaf and bark extracts of *C. oppositifolia*. For antibacterial activity methodology of [14] was used. For the bacterial strains i.e., *P. aeruginosa*, *K. pneumonia*, *E. faecalis*, *S. aureus*, *B. subtilis* and *E. coli*, the nutrient agar media was used. For antifungal activity the methodology of [15] was used. *Potato Dextrose Agar media* was used for antifungal activity.

Assessment of minimum inhibitory concentration (MIC)

Plant extracts of *C. oppositifolia* for MIC was investigated by broth-dilution method adapted by [16].

Statistical Analysis

All the statistical analysis was done utilizing Microsoft excel 2010.

Results and discussion

To checked the antimicrobial activity of the plant material i.e. *Colebrookea oppositifolia* Smith, extracts were prepared. Macerated and their % yield was intended before further analysis, to provide glance of the economical perspective of the complete practice. The phytochemical screening the plant extracts of *C. oppositifolia* Smith was also achieved. Results showed the presence and absence of secondary metabolites (Table 1 & 2).

Phytochemical recognition key

– = Absent, + = Present in minimal quantity, ++ = Existing in moderate quantity whereas, +++ = Present in noticeable amount. Six bacterial strains including three gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*) and three gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) were used for the estimation of antimicrobial activity of bark and fruit of *Cholebrookia oppositifolia* Smith. All of these bacteria were pathogenic. Furthermore, strains of fungal i.e., *A. oryzae* and *A. niger*, selected for antifungal prospective.

We also used some standard discs, to find out the receptiveness of the microbial strains. Which is used as the test organisms (Tables 3 & 4). According to these standards, the specimens were categorized as vulnerable, transitional, and resistant prone. Leaf and bark of *C. oppositifolia* produced different Zone of Inhibition in response of bacterial strains was recorded in mm. Zone of Inhibition was produced by the p. ether extract of leaf showed maximum results i.e. 18±0.6 mm against *B. subtilis*. Whereas, bark extract in p. ether showed maximum Zone of Inhibition i.e. 18±0.8 mm against *S. aureus* and 18±2.2 mm against *P. aeruginosa*. The leaf of *C. oppositifolia* Smith had displayed activity under the range of 0±0 mm to 18±0.6mm. Bark had exhibited the potential under the range of 0±0 mm to 18±2.2mm.

Table 1. Phytochemical evaluations of Bark extracts of *C. oppositifolia* Smith

Plant Part	Solvent	Presence / Absence of Phytochemical Constituents							
		Alkaloids	Steroids	Tannins	Flavonoids	Glycosides	Terpenoids	Cardiac Glycosides	Saponens
Bark	P. Ether	++	–	–	–	–	+	+	+++
	Chloroform	+	+	–	++	–	+++	+	++
	Methanol	+++	++	+	–	+	+	–	–
	Aqueous	–	–	+	+	–	–	–	–

Table 2. Phytochemical evaluations of Leaves extract of *C. oppositifolia* Smith

Plant Part	Solvent	Presence / Absence of Phytochemical Constituents							
		Alkaloids	Steroids	Tannins	Flavonoids	Glycosides	Terpenoids	Cardiac Glycosides	Saponens
Leaves	P. Ether	+++	+	–	+	+	–	–	++
	Chloroform	+	++	–	–	+	–	–	+
	Methanol	++	+	–	–	++	–	–	+
	Aqueous	–	–	–	+	–	–	–	–

Table 3. Zone of Inhibition (mm) produced by the bacterial strains against standard antibiotic discs

Antibiotic standard disc	Conc (µg)	Zone of inhibition (mm)					
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. faecalis</i>	<i>B. subtilis</i>
Methicillin	15	8±0.6	9±1.2	9±0.5	8±0.5	8±0.7	8±0.5
Amoxicillin	30	8±1.5	9±1.0	8±1.0	9±1.3	–	11±1.0
Azithromycin	16	14±2.6	14±1.3	10.5±0.7	11±0.5	10±1.5	12±1.5
Ampicillin	10	9±1.7	8±2.0	9±1.2	8±1.0	7±0.5	8±0.5
General Response		Resistant	Resistant	Resistant	Resistant	Resistant	Resistant

Table 4. Zone of Inhibition (mm) of fungal strains against the standard antifungal discs

Antifungal standard disc	Concentration (µg/ml)	Zone of inhibition (mm)	
		<i>A.niger</i>	<i>A.oryzae</i>
Nystatin	99.9	23±2.1	30±1.6
Tezole	99.9	23±0.6	67±2.6
Fungivin	99.9	16±3.5	40±1.8
General Response		Intermediate	Intermediate

The extract of leaf had showed supreme potential in contrast to *S. Aureus* compared to the bark with the petroleum ether and aqueous, as shown in (Figure 2). Whereas, chloroform extracts of leaf and bark, mutually had displayed nearly

muchalike potential with little difference. The potential exhibited by both parts was better as compared to standard discs. Theleast potency was showed by the methanolic extracts of both parts (Table5).

Figure 2. Graphical representation of the Zone of Inhibition against *S. aureus*

Table 5. The Zone of Inhibition produced by both parts of *C.oppositifolia* against the bacterial strains was documented in mm

Plant Part	Solvents	Zone of Inhibition (mm)					
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. faecalis</i>	<i>B. subtilis</i>
Leaf	P. ether	15±1.6	14±0.5	14±1.2	14±1.0	16±2.0	18±0.6
	Chloroform	13±0.8	12±0.5	12±0.5	14±0.7	14±1.5	12±0.3
	Methanol	10±0.8	11±0.6	10±0.3	12±0.6	11±0.7	13±0.8
	Aqueous	17±0.3	17±0.3	16±1.0	15±0.3	15±0.5	17±1
Bark	P. ether	18±0.8	15±0.3	18±2.2	17±2.0	13±0.4	15±0.3
	Chloroform	11±0.8	10±0.3	10±0.3	13±0.5	12±0.4	11±0.6
	Methanol	11±0.8	11±0.5	11±1.2	10±1.0	16±0.7	10±0.5
	Aqueous	16±0.5	17±0.3	15±2.8	11±0.6	15±0.6	17±0.3

Both leaf and bark extracts had showed marvelous potential against *E. coli* as well with the extracts in distilled water displaying the record potential in response of the specific bacterial strain (Figure 3). The p. ether extracts of both parts had showed also better results against *E. coli*.

The leaf extract had exhibited the record potential against *P. aeruginosa* as well. With the petroleum ether being the most effective one. The aqueous extracts of both parts of *C. oppositifolia* Smith had showed also satisfactory results against the particular bacterial strains (Figure 4).

Figure 3. Graphical representation of the Zone of Inhibition against *E. coli*

Figure 4. Graphical representation of the Zone of Inhibition against *P. aeruginosa*

Petroleum ether extract of bark (17 ± 2.0 mm) and aqueous extract of leaf (15 ± 0.3 mm) exhibited maximum potential against *K. pneumoniae*. Petroleum ether leaf extract and chloroform leaf extract had showed somewhat similar results against particular strains of bacteria i.e., 14 ± 1.0 mm and 14 ± 0.7 mm respectively. The activity displayed by both parts was greater, as compared to antibiotic discs (Figure 5). The petroleum ether extract of leaf (16 ± 2.0 mm) and methanolic extract of bark (16 ± 0.7 mm) showed maximum potential against *E. faecalis*. Aqueous extract of both leaf and bark of *C. oppositifolia* Smith exhibited somewhat similar and

satisfactory results against *E. faecalis* i.e. 17 ± 1 mm and 17 ± 0.3 mm. Whereas, chloroform bark extract and methanolic leaf extract shown minimal potential. The potential showed by both parts was better as compared to antibiotic discs which were used as standard (Figure 6).

The leaf extract had showed incredible activity against *B. subtilis*, as well with the extracts soaked in petroleum ether represented the maximum potential i.e., 18 ± 0.7 against the particular bacterial strain. The aqueous extracts of both parts had expressed significant activity against *B. subtilis* (Figure 7).

Figure 5. Graphical representation of the Zone of Inhibition against *K. pneumonia*

Figure 6. Graphical representation of the Zone of Inhibition against *E. faecalis*

Figure 7. Graphical representation of the Zone of Inhibition against *B. subtilis*

MIC Assay

Themethanolic extracts were only employed for the MIC estimation of *C. oppositifolia* Smith. The leaf extract repressed the growth of *S. aureus* efficiently while the bark extract

showed maximum activity against *E. coli* (Table 6).

Methanolic extract inhibited growth of *S. aureus* very effectively and MIC value was 0.49 ± 0.01 at conc. of 0.9 mg/mL and 0.10 ± 0.5 at conc. of 0.8 mg/mL (Figure 8).

The bark extract contained the significant potential against the all microbes, so we can say the bark extracts were more effective than leaf extracts. The bark and leaf extracts

of *C. oppositifolia* Smith were very effective against *E. coli*, their MIC values are 0.10 ± 0.33 and 0.09 ± 0.3 at conc. of 0.9 and 1 respectively (Figure 9).

Table 6. MIC values (mg/mL) exhibited by leaf and bark of *C. oppositifolia* against Gram-positive and Gram-negative bacterial strains.

Plant Part	<i>S. aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>K. pneumoniae</i>		<i>E. faecalis</i>		<i>B. subtilis</i>	
	Conc.	MIC	Conc.	MIC	Conc.	MIC	Conc.	MIC	Conc.	MIC	Conc.	MIC
Leaf	0.9	0.49 ± 0.01	0.9	0.10 ± 0.33	0.8	0.13 ± 0.7	0.9	0.09 ± 0.2	0.8	0.14 ± 0.5	0.7	0.10 ± 0.3
Bark	0.8	0.10 ± 0.5	1	0.09 ± 0.3	0.9	0.15 ± 0.5	0.8	0.10 ± 0.3	0.8	0.12 ± 0.7	0.8	0.11 ± 0.3

Figure 8. Graphical representation of the MIC values against *S. aureus*

Figure 9. Graphical representation of the MIC values against *E. coli*

The bark extract displayed supreme potential in response of *P. aeruginosa*. The leaf extract were less effective than bark extract (Figure 10). MIC values estimated for both parts were 0.13 ± 0.7 at 0.81 mg/mL and 0.15 ± 0.5 at 0.9 mg/mL correspondingly.

The *K. pneumoniae* was stated to be maximum delicate against the bark extract. When all methanolic extracts of *C. oppositifolia* Smith during analysis were taken to consideration. The bark extract were more active than leaf extracts (Figure 11). Actual MIC values estimated for both parts were 0.09 ± 0.2 at 0.9 mg/mL and 0.10 ± 0.3 at 0.8 mg/mL correspondingly.

The bark extract showed maximum potential against *E. faecalis* then leaf extract. The bark extracts were extra effective as compared to leaf extracts in that case. MIC values estimated for both parts against *E. faecalis* were 0.14 ± 0.5 at 8.0 mg/mL and 0.12 ± 0.7 at 8.0 mg/mL correspondingly (Figure 12).

The *B. subtilis* was stated to be the maximum resistant, against the methanolic extract of bark. Whereas, leaf extracts were extrareal than bark extracts. MIC values assessed for both parts of *C. oppositifolia* Smith against *B. subtilis* were 0.10 ± 0.3 at 0.7 mg/mL and 0.11 ± 0.3 at 0.8 mg/mL subsequently (Figure 13).

Figure 10. Graphical representation of the MIC values against *P. aeruginosa*

Figure 11. Graphical representation of the MIC values against *K. pneumoniae*

Figure 12. Graphical representation of the MIC values against *E. faecalis*

Figure 13. Graphical representation of the MIC values against *B. subtilis*

The antimycotic potential of the *C. oppositifolia* Smith was estimated using two fungal strains i.e. *A. niger* and *A. oryzae*. The extracts had shown variable potential against these fungal strains with the extreme potency being showed by the leaf extracts soaked in distilled water. However, petroleum ether, chloroform and methanolic extracts had showed minimal potential against these fungal strains (Table 7).

The *A. niger* was additional sensitive near the plant extracts when equated to the *A. oryzae*. The supreme potential against the *A. niger* had showed by the aqueous extract of leaf. The petroleum ether extract of leaf had displayed less potential as compared to the petroleum extract of bark against the *A. niger* (Figure 14; Table 8).

The *A. oryzae* strain had presented resistant towards the plant extracts in comparison to *A. niger*. The supreme potential against the respective fungal strain was showed by the distilled water extract of both parts. The extract macerated in methanol had showed resistance compared to other extracts. The potential exhibited by the bark extract of *C. oppositifolia* Smith in chloroform was greater compared to leaf extract (Figure 15 & 16).

The extracts macerated in the methanol were also utilized for the MIC estimation. The leaf extract had exhibited maximum potential against *A. niger* with the MIC value 0.55 ± 0.5 at 0.9 mg/mL whereas, bark extract showed 0.50 ± 0.5 MIC value at 0.8 mg/mL (Table 8).

Table 7. Zone of Inhibition exhibited by leaf and bark of *C. oppositifolia* Smith against fungal test organisms

Plant Part	Solvents	Zone of Inhibition	
		<i>A.niger</i>	<i>A.oryzae</i>
Leaf	P. ether	10±0.3	15±0.3
	Chloroform	14±0.6	16±0.6
	Methanol	14±0.6	17±0.9
	Aqueous	28±0.3	25±0.6
Bark	P. ether	14±0.3	15±1.2
	Chloroform	15±0.9	15±0.7
	Methanol	16±0.9	20±1.2
	Aqueous	24±0.6	24±1.5

Table 8. MIC values (mg/mL) exhibited by leaf and bark of *C. oppositifolia* against fungal test organisms

Plant Part	<i>A. niger</i>		<i>A. oryzae</i>	
	Conc.	MIC	Conc.	MIC
Leaf	0.9	0.55±0.5	0.8	0.11±0.3
Bark	0.8	0.50±0.5	0.8	0.21±0.5

Figure 14. Graphical representation of the Zone of Inhibition against *A. niger*

Figure 15. Graphical representation of the Zone of Inhibition against *A. oryzae*

Figure 16. Zone of inhibition produced by: (a) Aqueous leaf extract against *A. niger*(b) *P. ether* bark extract against *B. subtilis*(c) Aqueous leaf extract against *A. oryzae* (d) Aqueous leaf extract against *E. coli* (e) Aqueous leaf extract against *E. faecalis*(f)MeOH leaf extract against *E. faecalis*(g) Aqueous leaf extract against *K. pneumonia* (h) Aqueous bark extract against *E. faecalis*

Discussion

The highest yield obtained from the leaf of *C. oppositifolia* Smith was (17.13%). The highest % extraction of yield of the leaf of *C. oppositifolia* Smith was 7.73% which was nearly similar to the yield extract recorded by [17] during the assessment of some Indian plants with their ethno botanical values for their microbial potential. The lowest yield got in leaf extracts of *C. oppositifolia* Smith was 2.6% in methanol which was in accord to the yield extract gained from the plant (leaf) extract of lamiaceae family i.e. *Mentha longifolia* (L.) Huds. reported by [18] during screening of some selected medicinal important plants extracts for *In vitro* antimicrobial activity.

The phytochemical screening of *C. oppositifolia* Smith showed the occurrence of alkaloids, steroids, terpenoids, saponins, glycosides, tannins, reducing sugar, flavonoids and cardiac glycosides in sufficient amount.

Two types of bacterial strains, i.e. gram positive and gram negative were used for

investigation, in spite of their fundamental resemblances stated by [19].

The tendency of the antibacterial action shown by the extracts of *C. oppositifolia* Smith was nearly similar to that recorded by [20] on water and methanolic extracts from different South African plants. The determined potential was given by leaf extract of plant. The bark extract had providing good to reasonable potential. However, the extracts of leaf and bark had showed almost alike activity with minute differences.

Within solvents overall, the petroleum ether extracts of *C. oppositifolia* Smith had extraordinary antibacterial potential in contradiction of all the organisms experienced during investigation. The maximum potential exhibited was 18 ± 2.2 mm. Similar results were reported by [2] during analysis of antimicrobial and antioxidant activities of *Chenopodium ambrosioides*. This distinct activity had provided the evidence that the non-polar fractions i.e., petroleum ether were vigorous

in their activity compared to the relatively polar fractions i.e., methanol by [21].

The further amplification near the assessment of the antibacterial potential of *C. oppositifolia* Smith was providing by adopting microbroth dilution assay to estimate MIC value. And in this technique, methanolic extracts were only subjected to the MIC assay, because of its potential for the steady extraction of constituents got antibacterial potential. The MIC value of methanolic extracts of the plant under observation range from 0.09 ± 0.2 to 0.49 ± 0.01 mg/mL.

The MIC potential displayed by the *S. aureus* was estimated to be $\geq 0.10 \pm 0.5$ mg/mL. Furthermore, the MIC activity showed against *E. coli* was estimated to be $\geq 0.09 \pm 0.3$, against *P. aeruginosa* was $\geq 0.13 \pm 0.7$, against *K. pneumoniae* was $\geq 0.09 \pm 0.2$, against *E. faecalis* was $\geq 0.12 \pm 0.7$ and $\geq 0.10 \pm 0.3$ against *B. subtilis*. The MIC evaluations against the bacterial strains provided the evidence that the bark extract was more effective against *P. aeruginosa* followed by *S. aureus*. This results because of the fact that the bark was additional developed and ripe than leaves and may comprise some pigments and other composites which have been documented to inhibit with the antimicrobial activity of extracts stated by [22].

MIC efficiency for the fungal strain of the *A. niger*, was assessed to be within the range of 0.50 ± 0.5 to 0.55 ± 0.5 mg/mL, and *A. oryzae* was valued to be within the range of 0.11 ± 0.3 to 0.21 ± 0.5 mg/mL. So, it was clearly established that the potential of the plant against fungal strain was greater in *A. niger* than *A. oryzae* parallel findings observed by [22] during investigation of antimicrobial activity of *Tamarindus indica* Linn. and [23] screened the antimicrobial and antioxidant activities of *Cirsium wallichii* DC.

Conclusion

On the basis of phytochemical and antimicrobial screening of *Colebrookea oppositifolia* Smith, the results showed that the leaf and bark extracts had provided

excellent results against selected bacterial and fungal strains. The MIC assay had further provided the antimicrobial potential of plant's extracts. Thus, it can be concluded that all results are significant on the basis of increasing the concentration of the extracts. The antimicrobial activity is may be due to the presence of secondary metabolites i.e., alkaloids, flavonoids, tannins, sterols etc.

Authors' contributions

Conceived and designed the experiments: M Ajaib, Guide and wrote the article: S Abid, Performed the experiments: M Anjum, Q Noshad & MA Iqbal, Analyzed the data: M Ajaib & MF Siddiqui.

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