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Antioxidant, antibacterial and antifungal potential study of *Salvia macrosiphon* Boiss. stem extracts

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Abstract: The aim of present research work was to evaluate the *Salvia macrosiphon* Boiss. of Lamiaceae (mint family), using biochemical and biological assays. Plant's phytochemicals extraction was performed in methanol, butanol and water by mechanical shaking process. TPC and TFC were determined by Folin-Ciocalteu and aluminum chloride colorimetric procedures, respectively. The highest TPC (99.61±3.45 mg GAE/g) and TFC (234.72±7.12mg CE/g) were obtained in butanol and methanol, respectively. Regarding the antioxidant potential methanol extract showed the highest DPPH° scavenging potential (78.0±2.0%) and reducing activity (0.923±0.020 absorbance). The antibacterial activity of butanol extract against *P. aeruginosa* were found highest (ZOI = 23±2.00 mm). Antifungal study of methanol extract showed the ZOI (11 ±0.67mm) against *F. brachygibbosum*. The results revealed that the methanol stem extract of *S. macrosiphon* bear significant medicinal value and could be used for formulating phytomedicines and food preservers.

Keywords: *Salvia macrosiphon*, antioxidant, antibacterial, antifungal.

INTRODUCTION

Nature has endowed us with a wide variety of potentially active medicinal plants. The development of pharmaceutical industry merely depends on the characterization and medicinal evaluation of the isolated compounds from natural sources (Sahar *et al.*, 2013). The medicinal potential of plant materials are evaluated on the basis of their total phenolic contents (TPC), total flavonoid contents (TFC), antioxidant potential, antimicrobial potential or inhibition of lethal mechanisms in human body (Asghar *et al.*, 2016,Abbas *et al.*, 2021). *Salvia macrosiphon* Boiss. belongs to genus Salvia, which is a largest genus of Lamiaceae or mint family with estimated 700 to 1000 species of herbaceous perennials, and shrubs. It is native to southwest of Pakistan (Baluchistan), southern Europe and the Mediterranean areas. Hundreds of species of genus *Salvia* are well known for medicinal applications to cure diverse ailments such as chronic pain, inflammatory, cardiovascular and cerebrovascular diseases. It also used as nutritional spices. All species of genus *Salvia*, are famous for their pharmaceutical properties (Balaei-Kahnamoei *et al.*, 2021).

S. macrosiphon first gained the fame due to presence of polyphenolic compounds such as apigenin, luteolin, salvigenin, eupatorin and rosmarinic acid. These compounds were isolated from essential oil of its areal part, and reported as anti-acetyl cholinesterase, cytotoxic and antioxidant effects. The seeds extract of the plant also reported as a source of antibacterial and antioxidant agents (Gohari *et al.*, 2011). However, few research reports on this specie revealed that there is a dare need to address the medicinal values of other parts such as stem. The aim of present study was to explore the TPC, TFC, antioxidant and anti-microbial activities methanol, butanol and aqueous extracts of *S. macrosiphon* Boiss.

MATERIALS AND METHODS

Chemicals

All the chemical used to expedite this study were of analytical grade purchased either from Sigma-Aldrich (Germany) or Alfa-acer. Methanol, butanol, aluminium chloride ($AlCl_3$), Folin-Ciocalteu reagent, gallic acid ($C_7H_6O_5$), catechin ($C_{15}H_{14}O_6$,), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid ($C_6H_8O_6$) and Muller hinton agar were of Sigma-Aldrich.

Sample collection and identification

Plant was collected from District Quetta, Baluchistan south western region of Pakistan, and identified by plant taxonomist Prof. Dr. Rasool Bakhsh Tareen (Department of Botany, University of Baluchistan, Quetta) and SM-06-BUH specimen number was allocated to save in herbarium of the University of Quetta. The whole plant was washed with distilled water and dried under shade. The stem of the plant was grinded with grinder and then passed through 80 mesh sieve to achieve homogeneous sample. The sample was then stored in reduced air environment for using extraction purposes.

Extract preparation

The plant sample was extracted in methanol, butanol and water solvents using mechanical shaking process. Briefly, 50g of dried sample was soaked in 500 mL of each

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solvent, separately. The extraction was carried out at room temperature for 8h at 200 rpm, using orbital shaker followed by filtration, concentrate using rotary evaporator at reduced pressure. The crude extract weighed to determine the percentage yield using the formula given below. The samples were then stored at -4°C for further analysis.

$$\text{Yield (\%)} = \frac{\text{Weight of Powdered Sample (g)}}{\text{Weight of Solvent Free Extract (g)}} \times 100$$

Determination of total phenolic content

TPC were determined following the method reported by Asghar et. al. (2016). Briefly, the protocol was performed by mixing 1mL of tested extract of different concentrations (15 to 120 mg/mL) with 1mL of freshly prepared folin-ciocalteu reagent. After 5 min, 5 mL sodium carbonate solution (106g/L) was added, make the reaction volume 10 mL using distilled water followed by vigorously shaking. The reaction mixture was then allowed to stand at room temperature for 90 min in dark. Absorbance was recorded in triplicate at 765 nm by using spectrophotometer. Different gallic acid contractions (15 to 120 mg/mL) were used to calibrate the curve and reference standard. Concentration of TPC was calculated in term of mg gallic acid equivalents per gram of dry sample extract (mg GAE/g).

Determination of total flavonoid contents

TFC were determined following the method reported by Asghar et. al. (2016). The method is carried out by the addition of an aliquot of 0.25 mL sample (31.25 to 250 mg/mL) into 0.75mL of distilled water in a test tube followed by the addition of 0.15mL of5%sodium nitrite solution. The whole reaction mixture was incubated for 5 min at room temperature; then added 0.3mL of 10% (w/v) aluminum chloride solution and again allowed to incubate for another round of 5 min and finally added 1 mL of 1M sodium hydroxide solution. The absorbance was recorded at 510nm by using UV-visible spectrophotometer. Catechin was used as standard control to produce calibration curve. TFC was expressed in term of mg of catechin equivalent/g of dry mass of extract (mg CE/g).

In vitro antioxidant assays

The antioxidant potential of all extracts and the standard were measured according to the protocol reported earlier using DPPH free radical scavenging and reducing power assay (Asghar *et al.*, 2016).

DPPH free radical scavenging assay

In this one step procedure, an aliquot of 100µL solution of extract of different contractions (62.5-500mg/mL) were separately added to 1mL freshly prepared methanol solution of 0.1mM DPPH° in a test tube followed by 30 min incubation interval at room temperature (RT) in dark. The absorbance of each reaction mixture was recorded with spectrophotometer at 517 nm in triplicates. The percent inhibition of stable DPPH free radical was determined using the following formula;

$$\%\ \text{Inhibition of DPPH}^{\circ} = \frac{(A_{\text{initial absorbance}} - A_{\text{final absorbance}})}{A_{\text{initial absorbance}}} \times 100$$

Reducing power assay

The procedure was carried out by the addition of an aliquot of 1mL plant extract (125- 1000 µg/mL) in to 2 mL mixture of 0.2M sodium-phosphate buffer of 6.6 pH and 1% potassium ferricyanide solution of equal volume. The whole mixture was then shaken gently and incubated at 50°C for 20 min. Following the incubation period 1 mL aliquot of 10% trichloroacetic acid was added to stop the further reaction. The mixture of solution was centrifuged at 3000 rpm for 10 min. Supernatant was separated and a volume of 2.5mL was mixed with 2.5mL distilled water followed by the addition of 500µL of 1% aqueous solution of $FeCl_3$. After gentle shaking the absorbance of the mixture was noted at 700 nm.

Screening of antibacterial activity

The antibacterial potential of *S. macrosiphon* stems extracts were examined against gram positive *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and five gram negative bacteria; *Escherichia coli* (*E. coli*) ATCC 25922, *Klebisella pneumonia* (*K. pneumonia*) ATCC 15380, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Acinetobactor baumannii* (*A. baumannii*) ACTT 17978, and *Salmonella typhi* (*S. typhi*) SSFP(4s) which were obtained from clinical setup. The antibacterial activity was assessed using agar well diffusion method (Gonelimali *et al.*, 2018). The Mueller Hinton (MH) agar was used as medium and preparation of petri plats. All tested bacterial isolates were incubated at 37 °C for 24 h. The Molten MH agar was added in sterile plate, after 20 min MH agar was solidified. The surface of solidified media was swabbed with tested strains and five wells were formed with cork borer at each plate. Forty milliliter extract solution of four different concentrations (0.5 - 4 mg/mL) were poured separately into agar well. Ciprofloxacin with 5µg/40 mL concentration was also added in triplicate wells as standard antibacterial agent. The petri plates were then left for 1 h at room temperature for possible diffusion of antibacterial agent into the medium followed by incubation for 48 h at 37°C. After completion of incubation time, the diameter of the zone of inhibition (ZOI) were measured in three directions and mean value was calculated as antibacterial potential.

Screening of antifungal activity

The fungal strains, *Aspergillus niger (A. niger;* MTCC 1344*)*, *Fusarium avenaceum (F. avenaceum;* ATCC 60644*)* and *Fusarium brachygibbosum (F. brachygibbosum)* were used to test antifungal properties. The antifungal activity of *S. macrosopin* stem extracts were performed using well diffusion susceptibility method (Jehan *et al.*, 2011). Briefly, sterilized nutrient

agar medium plates were swabbed with 18 – 24 h cultures of microbial inoculate (standard inoculum of 1-2×10^7 CFU/mL, 0.5 McFarland standard). Six wells each of 8 mm in diameter, were formed in the solidified media plate with sterilized cork borer followed by the addition of 40 µL extract contraction (4mg/mL). DMSO was used as negative control and Gentamycin was used as positive control. Then cultured plates were incubated at 37°C for 24 h. After incubation period the ZOI was measured in mm.

STATISTICAL ANALYSIS

Statistical method ANOVA was used to calculate mean and standard deviations.

RESULTS

Extraction yield

All the three solvents; methanol, butanol and water showed satisfactory percent yield that are 16.68, 17.38 and 14.33%, respectively. The maximum yield was obtained in butanol.

Total phenolic and flavonoid content

The highest TPC (99.6±1.31mg (GAE)/g extract) was obtained in butanol extract while TFC (234.7±2.62mg (CE)/g extract) obtained in methanol extract. Summary of complete results is shown graphically in fig. 1.

DPPH free radical scavenging activity

The antioxidant capacity of *S. macrosiphon* stem extract in methanol showed highest free radical scavenging potential (78.00±2.00%); followed by butanol and water extracts as shown in fig. 2a. The standard antioxidant BHT showed 91.66±1.52% free radical scavenging potential.

Reducing power activity

Reducing power activity was investigated as a function of absorbance which is directly proportional to absorbance. The results are shown in fig. 2b. In comparison with our results, standard antioxidant (ascorbic acid) however showed low absorbance (0.780±0.110) as compared to sample.

Antibacterial activity

Table 1 shows the summary of antibacterial potential of *S. macrosiphon* stem extracts at different concentrations. At 4 mg/mL extract concentration the methanol, butanol and water extract showed 19±1.00, 23±2.00 and 15.5±0.5 mm ZOI, respectively against *P. aeruginosa* bacteria. While against gram-positive *S. aureus* bacteria the methanol, butanol and water extracts showed 19±1.0, 10.5±1.00 and 16±1.04 mm ZOI, respectively.

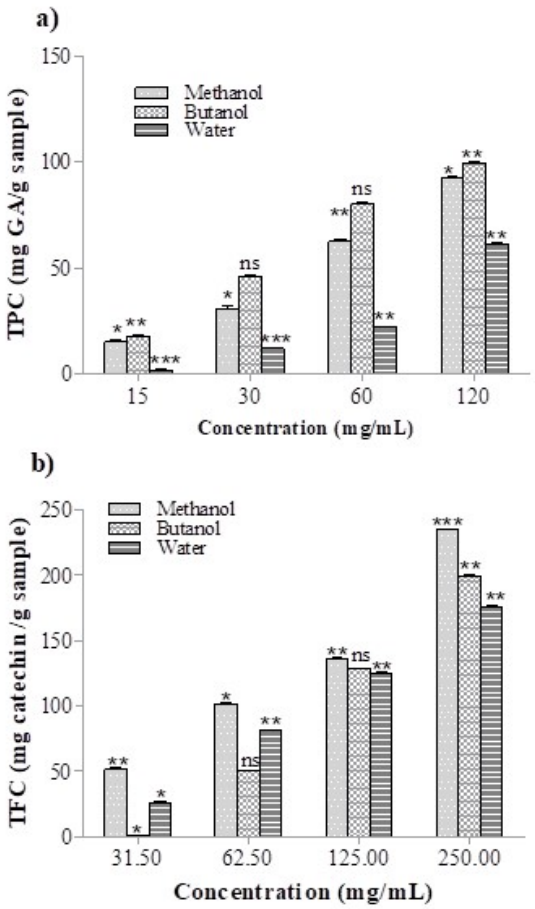

Fig. 1: TPC (a) and TFC (b) contents in *S. macrosiphon* stem extracts (n =3 ± S.D). Similar * showing non-significant difference.

Antifungal study

The results of antifungal activities are shown in table 2. The methanol extract possesses satisfactory potential to inhibit the growth of *F. brachygibbosum* (11.0±0.67 mm ZOI), followed by butanol and aqueous extracts against *A. niger* (10.5±0.58 mm ZOI) and *F. avenaceum* (9.0±0.51 mm ZOI), respectively. Gentamycin (standard) showed 17.5±1.675, 15.0±1.08 and 15±1.13mm ZOI against *F. brachygibbosum, A. niger* and *F. avenaceum*, respectively.

DISCUSSION

The present work was carried out to establish extraction yield, TPC, TFC, antioxidant, antibacterial and antifungal activities *Saliva macrosiphon* Boiss. The yield was obtained; 14.33, 16.68 and 17.38% in aqueous, methanol, and butanol medium, respectively. TPC and TFC are major secondary metabolites that impart medicinal impact to plants and they take part in numerous biochemical processes in living system as antioxidant and antimicrobial agents (Abbas *et al.*, 2021).

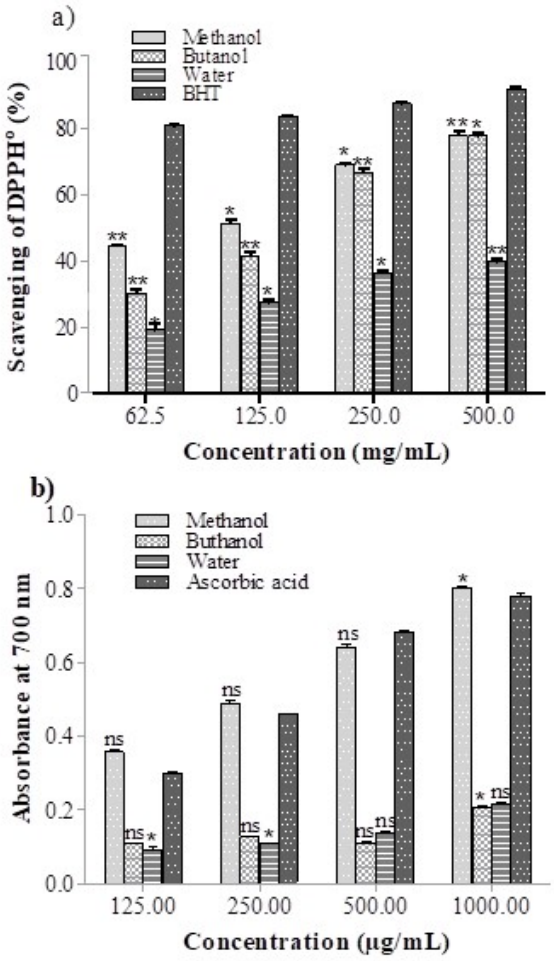

Fig. 2: Scavenging of DPPH˚ (a) and reducing power activity of *S. macrosiphon* stem extracts (b) (n =3 ± S.D). Similar * showing non-significant difference.

In this study, plant stem extracts showed pretty good value of phenolics (99.61±3.45 mg/g in butanol) and flavonoids (234.72±7.12 mg/g in methanol) which is higher than most of the reported results. The other two solvents such as methanol and water extracted less phenolics while butanol and water extracted less flavonoids. Antioxidant activities are performed either through reducing the target molecules or scavenging the free radicals such as hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot-}$) and nitric monoxide ($NO^{\cdot}$). Oxidative stress is known to trigger free radical production which make the living body vulnerable to initiate different diseases. Antioxidant estimation that how strongly the plant can be effective to scavenge free radicals and ultimately the diseases (Abbas *et al.*, 2021). The antioxidant capacity of all extracts were estimated by DPPH free radical scavenging and reducing power assay. Fig. 2a shows the DPPH free radical scavenging activity *S. macrosiphon* stem extracts. The results are compared with standard BHT (synthetic free radical scavenger).

Alcoholic extracts showed about 70% scavenging potential at 250 mg/mL concentration which looks very good. However, methanol extract showed good reducing potential of linoleic acid (82% at 1 mg/mL) which was higher than ascorbic acid reducing potential recorded under same reaction conditions. The antimicrobial activities of plant extract are basic studies to evaluate the medicinal potential of plant under studies. In past few decades, the overspread of fungal and bacterial infections and rise in bacterial resistance, draw the attention of phytochemists to combat the infection treatment challenge in clinical practice (Naqvi *et al.*, 2020). The antifungal potential of extracts, particularly alcoholic extracts showed satisfactory inhibition of fungal strains. In comparison to clinically approved synthetic drug, Gentamycin showed 15±1.0 mm ZOI in case of *A. niger and F. avenaceum* and 17.5±1.67 mm ZOI in case of *F. brachygibbosum*– However, alcoholic extracts with ZOI value 10.5±0.42 to 11±0.67 mm can be used for further refining to approach maximum values.

The antibacterial study marked the highest ZOI for butanol extract against *P. aeruginosa* followed by methanol extract against *S. typhi* (21±2.00 mm) and aqueous extract against *K. pneumonia* (21±1.52 mm). The standard antibacterial agent (Ciprofloxacin) showed 25±0.15 mm ZOI against *P. aeruginosa*, however against other strains it showed week inhibitory potential (table 2) which is mainly due to the bacterial resistance that has been now reported widely (Naqvi & Drlica, 2017,Naqvi, 2021). Recently published paper on antibacterial activities of areal part of *S. macrosiphon* methanol extract using *S. aureus* and *E .coli* in term of minimum inhibitory concentration (1.25 & 2.50mg/mL, respectively) showed good antibacterial activities (Balaei-Kahnamoei *et al.*, 2021). The results are in good agreement with our findings using *S. macrosiphon* stem extracts. The results of antibacterial study, however sufficient for good enthusiasm for moving ahead. In comparison to many studies reported in literature, *S. macrosiphon* extracts showed promising potential against bacterial growth inhibition.

CONCLUSION

S. macrosiphon Boiss. is a less explored shrub of Balochistan origin. *In-vitro* antioxidant, antifungal and antibacterial models were applied to evaluate its stem extracts in aqueous, methanol and butanol media. The extracts were found quite potent as antioxidant, antifungal and antibacterial. The antifungal activities, however found comparatively less effective as antioxidant and antibacterial. In conclusion, the results showed that the *S. macrosiphon* stem extracts in methanol could be investigated further as food preservative material and phytomedicine practice

Table 1: Antibacterial activities of *S. macrosiphon* stem extracts in methanol, butanol and water extracts.

Extraction Solvent/ Standard	Concentration (mg/mL)	Diameter of the zones of inhibition in mm					
		P. aerugin-osa	*E.* coli	*S. aureus*	*K. pneumonia*	*A. baumannii*	*S. typhi*
Methanol	*0.5*	8.0±1.00	9±0.81	9.0±3.0	-	9.5±0.86	8.0±1.00
	1	11±1.00	10±0.40	11±1.5	9.00±0.62	10±1.73	10±1.00
	2	13±1.73	14±0.40	12±0.66	10.5±1.00	8.5±0.75	13±2.10
	4	19±1.00	15±0.81	19±1.0	11.5±1.00	10±0.66	21±2.00
Butanol	*0.5*	17±1.00	10±2.00	10.5±1.0	8.5±0.5	9.0±1.0	11.0±0.5
	1	13±1.00	10±1.00	-	9±0.75	8.0±1.0	10.5±1.0
	2	18±1.00	9±1.00	-	9.5±2.17	9.0±1.0	12.0±1.0
	4	23±2.00	11±1.00	-	16±1.00	18±2.0	9.0±1.0
Water	*0.5*	7.5±0.38	7.5±0.28	7±0.28	7.5±0.5	-	-
	1	8.5±0.866	8±0.76	8±0.76	9±0.5	-	-
	2	14±0.76	9.5±0.28	9.5±0.28	10.5±0.5	-	-
	4	15.5±0.5	17±1.04	16±1.04	21±1.52	-	-
Ciprofloxacin	5	25±0.15	11±0.14	-	20±0.25	15±0.27	15±0.87

Values are expressed as mean± standard deviation, (-) = no result

Table 2: Anti-fungal activity of *Saliva macrosiphon* stems with different solvent extracts*;* methanol, butanol and water prepared using SSE method.

Extract/Drug/Technique	Diameter of the zones of inhibition in mm			
	Concentration mg/mL	*A. niger*	*F. avenaceum*	*F. brachygibbosum*
Water	4	8.5±0.39	9±0.51	10±1.75
Methanol	4	10.5±0.42	10±0.66	11±0.67
Butanol	4	10±0.58	10.5±1.0	9±0.51
Gentamycin	5µg/disk	15±1.08	15±1.13	17.5±1.67

Values are expressed as mean± standard deviation

REFERENCES

Abbas A, Naqvi SAR, Rasool MH, Noureen A, Mubarikand MS and Tareen RB (2021). Phytochemical analysis, antioxidant and antimicrobial screening of *Seriphidium oliverianum* plant extracts. *Dose-Response,* **19**(1): 1-9.

Asghar N, Naqvi SAR, Hussain Z, Rasool N, Khan ZA, Shahzad SA, Sherazi TA, Janjua RSA, Nagra SA, Zia-Ul Haq M and Jaafar HZ (2016). Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. *Chem. Cent. J.,* **10**(1): 1-11.

Balaei-Kahnamoei M, Eftekhari M, Ardekani MRS, Akbarzadeh T, Saeedi M, Jamalifar H, Safavi M, Sam S, Zhalehjooand N and Khanavi M (2021). Phytochemical constituents and biological activities of *Salvia macrosiphon* Boiss. *BMC Chem.,* **15**(1): 1-7.

Gohari AR, Ebrahimi H, Saeidnia S, Foruzani M, Ebrahimiand P and Ajani Y (2011). Flavones and Flavone Glycosides from *Salvia macrosiphon* Boiss. *Iran. J. Pharm. Res.,* **10**(2): 247-251.

Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chenand M and Hatab SR (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front. Microbiol.,* **9**(1639): 1-9.

Jehan B, Amjadand I and Mohammad S (2011). Antimicrobial potentials of Eclipta alba by well diffusion method. *Pak. J. Bot.,* **43**(Sp. Issue): 169-174.

Naqvi SAR (2021). 99mTc-labeled Antibiotics for Infection Diagnosis: mechanism, action and progress. *Chem. Bio. Drug Des.,* https://doi.org/10.1111/cbdd.13923

Naqvi SAR and Drlica K (2017). Fluoroquinolones asimaging agents for bacterial infection. *Dalt. Trans.,* **46**(42): 14452-14460.

Naqvi SAR, SMA Shah, L Kanwal, M Saeed, H Atta ul, J Nisar, Z Nisarand M Akram (2020). Antimicrobial and antihypercholesterolemic activities of *Pulicaria gnaphalodes. Dose-Response,* **18**(1): 1-9.

Sahar A, Naqvi SAR, Hussain Z, Nosheen S, Khan ZA, Ahmad DM, Asi MR, Saharand T and Shakeela N (2013). Screening of phytoconstituents, investigation of antioxidant and antibacterial activity of methanolic and aqueous extracts of *Cucumis sativus* L. *J. Chem. Soc. Pak.,* **35**(2): 456-462.