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PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF *PORTULACA OLERACEA* L. AGAINST MULTIPLE DRUG RESISTANT (MDR) PATHOGENS ISOLATED FROM CLINICAL SPECIMEN

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ABSTRACT

The present investigation was carried out for evaluation of antibacterial activity of *Portulaca oleracea* L. against multiple drug resistant (MDR) bacteria isolated from clinical specimen. As microorganism are becoming resistant to present day antibiotics, our study focuses on antibacterial activity and future prophylactic potential of the *Portulaca oleracea* L. The antibacterial activity of *Portulaca oleracea* L. were evaluated on MDR strains such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp., Enterococcus faecalis, Citrobacter freundii, Acinetobacter baumannii, Streptococcus pneumoniae, Enterococcus faecium and Enterobacter cloacae.* Antibacterial activity of five different solvent extracts (Methanol, acetone, ethanol, petroleum ether and n-Hexane) were prepared by using Soxhlet extractor. *In-vitro* antibacterial activity was performed by agar well diffusion method. The phytochemical anyalsis of leaves extract of *Portulaca oleracea* L. showed the presence of Saponins, Glycosides, Alkaloids, Flavonoids, Phenolic substance, Steroids, Di & Tri-terpenes and Tannins, Glycosides and Steroids. The maximum antibacterial activity of leaves of *Portulaca oleracea* L. found in methanolic extract. The highest zone of inhibition of methanolic leaves extract was found against *E.coli* (26 mm) followed by *S. aureus* (24 mm), *S. pneumoniae* (24 mm), *K. pneumoniae* (22 mm), *S. typhi* (22 mm) whereas in ethanolic extract maximum zone of inhibition was found against *S. pneumoniae* (22 mm), *E. coli* (20 mm), *S. aureus* (18mm), *C. freundii* (18 mm) and *K. pneumoniae* (18 mm). The lowest MIC

value was found in methanolic extract was 0.79 mg/ml against *S. aureus, E.coli* and *S. pneumoniae* alternatively the lowest MIC value of ethanolic extract was 1.56 mg/ml found against *S. aureus, E.coli, S. typhi, E. faecalis, A. baumannii* and *S. pneumoniae.*

KEYWORDS: MDR, Antibacterial Activity, *Portulaca oleracea* L., Soxhlet extractor.

INTRODUCTION

Antibiotic resistance is a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics; pathogens resistant to multiple antibiotics are considered *multidrug resistant* (MDR) or, more commonly, superbugs. Since its discovery, antibiotics are essential drugs to treat bacteria-producing infectious diseases. Many of the available antibiotics are no longer effective because of emerging resistance, which is mainly caused by inappropriate antibiotics used by prescribers, self-prescription by consumers, counterfeit dealers, etc. In addition, lack of standard treatment guidelines, training for prescribers and pressure from the pharmaceutical industry are important determinants influencing resistance acquisition. [1]

Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century, in particular as it pertains to pathogenic organisms. In the simplest cases, drug-resistant organisms may have acquired resistance to first-line antibiotics, thereby necessitating the use of second-line agents. Typically, a first-line agent is selected on the basis of several factors including safety, availability, and cost; a second-line agent is usually broader in spectrum, has a less favorable risk-benefit profile, and is more expensive or, in dire circumstances, may be locally unavailable. In the case of some MDR pathogens, resistance to second- and even third-line antibiotics is, thus, sequentially acquired, a case quintessentially illustrated by *Staphylococcus aureus* in some nosocomial settings. Some pathogens, such as *Pseudomonas aeruginosa*, also possess a high level of intrinsic resistance.

There were low levels of preexisting antibiotic-resistant bacteria before the widespread use of antibiotics. [2] Evolutionary pressure from their use has played a role in the development of multidrug-resistant varieties and the spread of resistance between bacterial species. [3] In medicine, the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics. [4] Given the alarming incidence of antibiotic resistance in bacteria of

medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Several screening studies have been carried out in different parts of the world.

Portulaca oleracea L. is a member of the Portulacaceae family with more than 120 different species. The name Portulaca is thought to be derived from the Latin "porto" meaning "to carry"and "lac" meaning milk, since the plant contains a milky juice; oleracea from Latin, meaning "pertaining to kitchen gardens", referring to its use as a vegetable. The use of this plant as a vegetable, spice and medicine has been known since the times of the ancient Egyptians and was popular in England during the middle Ages. [5] It has been cultivated in India and the Middle East and has been popular in Europe since the Middle Ages. *Portulaca Oleracea L* is an annual prostrate or spreading, succulent, branched herb of the Postulacacea family. *Portuleca oleracea L* is a summer annual which is grown as a vegetable in many parts of the world. Recent studies on *Portulaca oleracea L.*extracts have showed muscle relaxant activity, reduction in locomotors activity, increase in onset time of pentylenetrazole-induced convulsion [6], analgesic and ant-inflammatory effects. [7] It is used in Iranian folk medicine as a diuretic, vermifuge, antiscorbutic, antitussive, analgesic and in gastroesophageal reflusx. In traditional medicine, this plant is utilized as antivomiting, antibleeding, antihepatitis and in treatment of gastric mucosal diseases; in some middle east countries, it is considered beneficial for small tumors and inflammation, urinary disorders, liver obstruction and ulcers of mouth and stomach. Researchers have shown that this plant having antifertility effect, Antihyperglycemic activity, Antitumor activity, Antiulcer Activity.[8] There is report that, *Portuleca oleracea L* has the gastric antiulcer genic activity.[9]

This plant contains vitamins A, Vitamin C and some vitamin B and caretoinoids as well as dietary minerals such as magnesium, calcium, potassium and iron. It also contains two types of betalain alkaloid pigments, the reddish betacyanins and the yellow betaxanthins. Both of these pigment types are potent antioxidants and have been found to have antimutagenic properties. Many types of chemical compounds were present in this plant, including alkaloids, terpenoids, organic acids, coumarins, flavonoids, volatile oil and polysaccharides. [10] Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity.

MATERIALS & METHODS

1) Plant Materials

Medicinal plants and their parts were collected from different areas of Nagpur city. This plant then authentified from P.G. Department of Botany, R.T.M. Nagpur University, Nagpur. Leaves were collected washed with sterile distilled water and air dried at room temperature. Dried leaves were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers for further use.

2) Herbal preparations

The dried plant materials (20 gm) were extracted with 200 ml of each solvent separately by using Soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the Solvent. The solvents used for the study were methanol, ethanol, petroleum ether, acetone and n-hexane. The extracts were filtered and then concentrated to dryness. The extract were transferred to glass vials and kept at 4ºC before use. The extracts were dissolved in 20% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml. The stock solutions were stored in a refrigerator until needed.

3) Phytochemical analysis

The phytochemical screening of all the extracts was carried out to determine the presence of the following compounds; alkaloid, flavonoids, polyuronides, reducing sugars, cyanogenic glycoside, saponins, terpenes, anthracenosides, phytosterols and phenols as described below. [11]

3.1: Saponins (the Froth test)

2 ml of the extract was added to distilled water and shaken vigorously. A froth (foam) that persisted for more than 10 minutes indicated the presence of saponins.

3.2: Glycosides

To the solution of extract in glacial acetic acid few drops of ferric chloride and conc. H_2SO_4 are added and observed for reddish brown coloration at the junction of 2layers and bluish green color in upper layer.

3.3: Polyuronides / Polyamides

Ten milliliters of acetone was added to 2ml of the extract in a test tube. The appearance of a Precipitate indicated the presence of polyuronides.

3.4: Reducing sugars

Two milliliters of the extract was diluted in 2ml of distilled water and Fehling's solutions (A+B) added to the mixture. A brick red precipitate after standing in the heat or water bath indicated the presence of reducing sugars.

3.5: Alkaloids

Twenty milliliters of the alcohol extract was evaporated to dryness on a water bath. Five to ten milliliters of 10% hydrochloric acid (HCl) and $CHCl_3$ were added to the extract. Concentrated ammonia was added to the aqueous layer to obtain a pH of between 8 and 9. The solution was then extracted in a separating tube with chloroform or ether. The a polar solvent was evaporated to dryness in an evaporated dish in a water bath and the residue was dissolved with 5ml of HCl (2N) and the solution was divided into three separate test tubes. Two to three drops of Mayer's reagent was added to one and the same amount of Bertrand's reagent to the other, while the third test tube served as a reference. The appearance of an opalescent or yellow-white precipitate with the reagents indicated the presence of alkaloids.

3.6: Anthracenocides

Four milliliters of the extract was concentrated to 2ml with 2ml of 25% of ammonia solution added and shaken. A cherry red colour of the alkaline layer indicated the presence of emodols (aglycones of anthracenosides) in an oxidized form–Borntrager's reaction.

3.7: Flavonosides

Five milliliters of the extract was evaporated to dryness. The residue was dissolved in 2ml of 50% methanol by heating and 4 grams of metal magnesium and 6 drops of concentrated HCl added. A red solution indicated the presence of flavonoids, while an orange solution indicated the presence of flavones.

3.8: Phenolic substances

Two to three drops of 10% Ferric chloride solution was added to 5ml of extract in a test tube and observed. Dark Green color was develops indicated positive results.

3.9: Sterols and Triterpenes

Ten milliliters of the extract was evaporated to dryness. The residue was dissolved in 0.5ml of acetic aldehyde and 0.5ml of $CHCl_3$ added and transferred into a dry test tube. About two milliliters of concentrated sulphuric acid (H_2SO_4) was added to the bottom of the tube using a

pipette. A brownish red or violet ring at the contact zone of the two liquids indicated the presence of sterols and triterpenes. The greenish and brownish red (wine) nature of the supernatant indicated the presence of sterols and triterpenes respectively.

3.10: Test for Tannins

To 0.5 ml of extract solution 1 ml water and 1-2 drops of ferric chloride solution was added. Blue color was observed for garlic tannins and greenish black for catecholic tannin.

3.11: Test for amino acids

1 ml of plant extract add 2 ml of Ninhydrins. For positive results indicates forming purple color.

3.12: Test for proteins

1 ml of dilute extract add 1 ml of 5% $CuSO_4$ add 1% of 1ml of NaOH. Deep blue color indicates positive results.

3.13: Test for Saponin

To 50 mg powder and add 20 ml distilled water shake for 15 minutes. Forming 2 cm foam was produced in measuring cylinder indicated positive results.

4. Bacterial Isolates

Multiple drug resistant bacteria were isolated from different clinical specimen such as urine, blood, wound swabs/pus, cerebrospinal fluid and sputum. The MDR strains were identified on the basis of their morphology, cultural, biochemical characteristics as well as antibiotic susceptibility test. These all MDR bacteria were resistant to more than 10 antibiotics. The MDR strains used for the antibacterial activity were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp., Enterococcus faecalis, Citrobacter freundii, Acinetobacter baumannii, Streptococcus pneumoniae, Enterococcus faecium and Enterobacter cloacae.*

4: Determination of the potency of the herbal preparation

The agar diffusion method was used to investigate the antibacterial activity of the crude extracts. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterilized swab was aseptically dipped into the suspension. The dried surface of a Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface with bacteria. A sterilized cock borer of an internal diameter of about 6 mm was used to

punch holes in the medium and plant extracts were dispensed into the respective labeled holes. 20 % v/v DMSO was used as negative controls. Triplicates of each plate were made and the procedure was repeated for the other microorganisms. The plates were kept in the refrigerator for about 4 hours for complete diffusion of the extract and incubated at 37ºC for 24 hours. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with zone measuring scale.

5: Determination of minimum inhibitory concentration (MIC) of the crude extracts

MIC for each test organism was determined by following the modified agar well diffusion method. A twofold serial dilution of each extract was prepared by first reconstituting the dried extract (100 mg/ml) in 20% DMSO followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50mg/ml to 0.195 mg/ml. A 100 µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100µl of standardized inoculum (10^6cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37^0C for 24 hrs and observed for the inhibition zones. MIC, taken as the lowest concentration of the test extract that completely inhibited the growth of the microbe, showed by a clear zone of inhibition (>8mm), was recorded for each test organism. [12, 13]

RESULTS & DISCUSSION

Beyond the increasing prevalence of antibiotic resistance among pathogenic bacteria, undesirable side effects of some synthetic antibiotics add urgency to the search for new infection and fighting strategies, as well. Scientist and pharmaceutical industries have considered the medicinal plants as a good choice, because these natural resources have ordinary fewer side effects and are costless and more effective against broad spectrum of antibiotic resistant bacteria. The extracts of medicinal plants are used for their antibacterial, antifungal and antiviral properties in many parts of the world. [14] Plant species used in folk medicine are potential for discovering extracts with active biological compounds that have antibacterial activity. *Portulaca oleracea* L. plant is one among the most important plant extensively used in traditional medicine in India, the extracts of plant have antibacterial effect on multiple drug resistant bacteria. The result of phytochemical analysis was revealed that the presence of most of the phytochemical constituents [Table No.1]. The phytochemical anyalsis of methanolic leaves extract of *Portulaca oleracea* L. showed the presence of Saponins, Glycosides, Alkaloids, Flavonoids, Phenolic substance, Steroids, Di & Tri-terpenes and

Tannins whereas Glycosides and Steroids were absence in ethanolic extract compared with methanolic extract. The study conducted by Dhole JA (2011) also found the similar phytochemical results. [15] The similar study also conducted by Ramesh Londonkar (2011) showed the presence of all phytochemical substances. [16]

The antibacterial activity of *Portulaca oleracea* L. showed excellent zone of inhibition against tested multiple drug resistant bacteria. The highest zone of inhibition of methanolic leaves extract was found against *E.coli* (26 mm) followed by *S. aureus* (24 mm), *S. pneumoniae* (24 mm), *K. pneumoniae* (22 mm), *S. typhi* (22 mm) and *C. freundii* (20 mm). *P. aeruginosa, P. mirabilis, A. baumannii* and *E. faecium* showed 18 mm zone of inhibition in methanolic extract [Table No. 2]. The lowest MIC value of methanolic extract was 0.79 mg/ml found against *S. aureus, E.coli* and *S. pneumoniae* whereas inhibitory concentration at 1.56 mg/ml observed in *S. typhi, E. faecalis, C. freundii, A. baumannii and E. faecium* [Table No. 3]. Bakkiyaraj S et. al. (2011) showed methanol extract of *Portulaca oleracea* showed high activity against both Gram positive organisms *Bacillus subtilis* (20mm) and *Staphylococcus aureus* (15mm) and only active against one Gram-negative bacteria namely *Pseudomonas aeruginosa* (18mm). [17] These might be due to presence of triterpenoids, phenolic compounds, Carotenoids, steroids, ketones and tetra-triterpenoids azadirachtin. These results were similar to those reported by Monroe S et. al. The ethanolic extract also showed good inhibitory activity against tested bacteria. [18] The maximum zone of ethanolic extract was found against *S. pneumoniae* (22 mm), *E.coli* (20 mm), *S. aureus* (18mm), *C. freundii* (18 mm) and *K. pneumoniae* (18 mm). *S. typhi, E. faecium and P. aeruginosa, P. mirabilis, E. faecalis, E. cloacae* showed 16mm and 14 mm zone of inhibition respectively [Table No. 2]. The study done by Dhole JA (2011) showed that the ethanolic extract shows good inhibitory activity against *S. aureus* (31 mm) and *Pseudomonas aeruginosa* (29 mm) at concentration of 100mg/ml which found to be high when compared with the present study.[15] The another study conducted by Ramesh Londonkar (2011) also found that the ethanolic extract showed good inhibitory activity against Gram positive and Gram negative bacteria.[16] These might refer to the presence of coumarins, flavonoids and saponins as chemical components of these plants. [19] The lowest MIC value of ethanolic extract was 1.56 mg/ml found against *S. aureus, E.coli, S. typhi, E. faecalis, A. baumannii* and *S. pneumoniae* whereas highest MIC value was 6.25mg/ ml found against *K. pneumoniae, P. mirabilis, E. faecium* and *E. cloacae* [Table No. 3]. The acetone, n-hexane and petroleum ether extract showed moderate zone of inhibition shown in Table No. 2. The plant *Portulaca oleracea*

possesses several phytochemicals and has significant antibacterial properties. [20] The phytochemicals like alkaloids, saponins, flavonoids and phenolic compounds present in plants are responsible for many biological activities. [21] The ethanopharmacological exploration of plant species derived antimicrobial agents is needed for the production of safe and standardization of therapeutic drugs against harmful microbes. *Portulaca oleracea* L. extract possess a broad spectrum of antimicrobial activity against a panel of bacteria responsible for the most common bacterial diseases.

Table No. 1: Phytochemical analysis of *Portulaca oleracea* L.

S. No	Phytochemical analysis	*Portulaca Oleracea* L.	
		Solvents	
		Methanol	Ethanol
1.	Saponins	+	+
2.	Glycosides	+	-
3.	Polyamides	-	-
4.	Reducing Sugars	-	-
5.	Alkaloids	+	+
6.	Steroids	+	-
7.	Flavonoids	+	+
8.	Phenolic substance	+	-
9.	Di & Tri-terpenes	+	+
10.	Tannins	+	+
11.	Amino acids	-	-
12.	Proteins	+	+

Table No. 2: Antibacterial activity of *Portulaca oleracea* L. against multiple drug resistant (MDR) bacteria.

Plant Part used	Solvent	Zone of inhibition in mm											
		S. aureus	***E.coli***	***P. aeruginosa***	***K. pneumoniae***	***P. mirabilis***	***S. typhi***	***E. faecalis***	***C. freundii***	***A. baumannii***	***S. peumoniae***	***E. faecium***	***E. cloacae***
Leaves	Ethanol	18	20	14	18	14	16	14	18	17	22	16	14
	Methanol	24	26	18	22	18	22	15	20	18	24	18	16
	Petroleum Ether	12	10	8	8	7	8	9	8	10	10	10	8
	Acetone	16	18	12	12	10	12	12	12	11	12	12	11
	n-Hexane	8	8	10	8	9	10	8	8	8	8	8	8

NZ – No Zone

Table No. 3: MIC of *Portulaca oleracea* L. against Multiple drug resistant (MDR) bacteria.

Plant Part used	Solvent	Minimum Inhibitory Concentration in mg/ml											
		S. aureus	***E.coli***	***P. aeruginosa***	***K. pneumoniae***	***P. mirabilis***	***S. typhi***	***E. faecalis***	***C. freundii***	***A. baumannii***	***S. peumoniae***	***E. faecium***	***E. cloacae***
Leaves	Methanol	0.79	0.79	3.125	3.125	3.125	1.56	1.56	1.56	1.56	0.79	1.56	3.125
	Ethanol	1.56	1.56	3.125	6.25	6.25	1.56	1.56	3.125	1.56	1.56	6.25	6.25

CONCLUSION

From the above investigation it can be conclude that the extract of *Portulaca oleracea* L. can be considered as a resource for potential antimicrobial agents. However, the present findings may also supplement and strengthen the process of standardization and validation of herbal drugs containing active ingredients derived from the selected medicinal plants.

REFERENCES

1. Carlos F. Amabile-Cuevas. Antimicrobial Resistance in Bacteria. Horizon Biosciences.
2. Caldwell R, Lindberg D. (Eds.), Understanding Evolution [Mutations are random]. University of California Museum of Paleontology, 2011.
3. Hawkey PM, Jones AM. The changing epidemiology of resistance. J Antimicrob Chemother 2009; 64: 3-10.
4. WHO Global Strategy for Containment of Antimicrobial Resistance, 2002.
5. Okafor Izuchukwu Azuka, Ayalokunrin Mary B. and Orachu Lovina Abu. A review on *Portulaca oleracea* (Purslane) plant – Its nature and biomedical benefits. International Journal of Biomedical Research. 2014; 05(02).
6. Radhakrishnan R, Zakaria MNM, Islam MW. Neuropharmocological actions of portuleca oleracea L. subsp. Sativa(haw). J Ethanopharmocol 2001; 76: 171-176.
7. Chan K, Islam MW, Kamil M. The analgenic and anti-inflammatory effect of portuleca oleracea L subsp. Sativa (haw) Celak. J Ethanopharmacol, 2000; 73: 445-451.
8. Ivan A, Ross A. Text book of medicinal plants of the world, 2003.
9. Gholamreza Karmi, Hossein Hosseinzadeh and Negin Ettehad. Evaluation of the gastric antiulcerogenic effect of portuleca oleracea L extracts. Phytother, res, 2004; 18: 484-487.
10. Prashant KL, Jadav H, Thakurdesai P, Nagappa AN. The cosmetic potential of herbal extracts. Nat prod Radiat, 2005; 4:351.
11. Sofowora H. Screening Plants for Bioactive Agents in: Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Sunshine House, Ibadan. Nigeria, 2nd Ed, 1993; 134-156.
12. Aneja KR, Joshi R, Sharma C. Antimicrobial activity of Dalchini (*Cinnamomum zeyla nicum* bark) extracts on some dental caries pathogens. J Phar Res, 2009; 2(9):1370-2.
13. Nkere CK and Iroegbu CU. Antibacterial screening of the root, seed and stem bark extracts of *Picralima nitida.* Afr J Biotechnol, 2005; 4: 522-526.
14. Hassawi D. and Kharma A. Antimicrobial activity of some medicinal plants against *Candida albicans.* J. Biological Sci, 2006; 6: 109-114.

15. Dhole JA, Dhole NA, Lone KD and Bodke SS. Preliminary Phytochemical Analysis and Antimicrobial Activity of Some Weeds collected from Marathwada Region. Journal of research in Biology, 2011; 1: 19-23.
16. Ramesh Londonkar and Hanumantappa B. Nayaka. Phytochemical and Antimicrobial Activities of Portulaca Oleracea L. Journal of Pharmacy Research, 2011; 4(10): 3553-3555.
17. Bakkiyaraj S and Pandiyaraj S. Evaluation of potential antimicrobial activity of some medicinal plants against common food-borne pathogenic microorganism. International Journal of Pharma and Bio Sciences Vol 2/Issue 2/Apr-Ju 2001; 484- 491.
18. Monroe S and Polk R. Antimicrobial use and bacterial resistance. Curr Opin Microbial 3.96-501, 4, *Portulaca oleracea* by a single cell bioassay system" Phytotherapy research, 2000; 14(5): 329-332.
19. Holetz FB, Pessini GL, Sanches N, Cortez DA, Nakamura CV and Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 2002; 97: 1027-1031.
20. Bongoh, Moochang and Jun Hwang. Detection of antifungal activity in Portulaca oleracea by a single cell bioassay system. Phytotherapy research, 2000; 4(5):329-332.
21. Rabe Tandvan Staden J. Isolation of an antimicrobial sesquiterpenoid from Warbugie salutaris. J. *Ethnopharcol,* 2000; 93:171-174.