Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

POLAR AND NON POLAR SOLVENT EXTRACTION AND PHARMACOLOGICAL EVALUATION OF FOUR DIFFERENT PARTS FROM *BRASSICA NIGRA* (KOCH.) PLANT

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DOI: 10.7897/2277-4572.02333
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 15/04/13 Revised on: 20/05/13 Accepted on: 29/05/13

ABSTRACT
Profound research has been done on the medicinal value of *Brassica nigra* (BN) seeds, and the leaves of the plant have been investigated in this study. The methanol extracts of the leaves were subjected to several in vitro studies. Among the four parts of this experimental plant seed extracts, which showed better antimicrobial activity in the initial screening test, were selected for further investigations. Though a floral extract with chloroform solvent showed refusal activity against five tested bacteria. However, maximum zone of inhibition observed in seed extract with polar and non polar solvents such as Pet. Ether, Chloroform and methanol against *S. aureus* (17.90 ± 2.1), *P. mirabilis* (17.8±4.02), *E. aerogens* (15.71.4±0.2), *P. vulgaris* (14.7±2.8), *E. coli* (11.47±3.3). Comparison of the antimicrobial activities of the fractions with that of the crude extract indicated that the non-polar fractions in many cases are stronger in activity at the two concentration levels than the crude extract. Results of the present study indicated that seed extracts with polar and nonpolar solvents can be exploited for future antimicrobial drugs against selected pathogenic bacterial strain.
Keywords: *Brassica nigra*, MBC, MFC, MIC, Total activity, polar and nonpolar solvents

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents[1, 2]. Emergence of pathogenic microorganisms that are resistant/ multi resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs[3]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immunosupression etc.) and are major burning global issues in treating infectious diseases[4]. Although, pharmacological industries had produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi drug resistant microorganisms have exacerbated the situation[5,6]. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines[7]. Antimicrobials of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics[8]. Positive response of plant based drugs (less/ no side effects) might lies in the structure of the natural products which reacts with toxins and/or pathogens in such a way that less harm is done to other important molecules or physiology of host. It is because of this reason that drug designing studies nowadays have come up as new field of research. Antibacterial activity may vary between different strains of same species and moreover depends on the form that is used such as dried, fresh or extracted and also on the harvesting seasons and geographical area as well[9]. Very recently,[10] Alyaa, 2012 studied the antibacterial activity of oils extracts of *B. nigra* seeds on some bacteria isolated from plaque and healthy teeth in children (1-5) years. Still the similar kind of no other works has been done till today. Since the present work designed the objective was to study the pharmacological evaluate the four parts of the *B .nigra* plant and its pharmacological activity against chosen bacteria.

MATERIALS AND METHODS

Plant material and extraction procedure

Different parts of *B. nigra* (stem, leaf, and flowers) and *C. decidua* (root, stem, and fruits) were collected in the month of August from the western parts of India (Malankara Catholic College, Mariagiri) Kaliakkavilai THEN Voucher specimen was identified by Dr. Mary Sujin, CBP, from St. Xavier's College, Palayamkottai. Selected parts of this plant (Leaf, stem, root and seed) were separately shade dried, finely powdered using a blender, and subjected to extraction following the method of Palombo and Semple, 2001. Hundred grams of each finely powdered sample was Soxhlet extracted with 80 % hot methanol (500 ml) on a water bath for 24 h and filtered. Filtrate was re-extracted successively with petroleum ether (fraction- I), methanol (fraction II), and chloroform (fraction III) using separating funnel. Petroleum ether fractions were discarded as being rich in fatty substances. Methanol fraction of each of the samples was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h (for removal of bounded sugars from the flavonoids). Resulting mixture was filtered and filtrate was extracted with ethyl acetate in separating funnel. Petroleum ether extract thus obtained was washed with distilled water to neutrality fractions were dried and weighed. The extracts were stored at 4°C and were re-suspended in their respective solvents to get 10 mg/ml for antimicrobial assay.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC/MFC)

Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against test pathogens. Broth microdilution method was followed for determination of micro titer plate that showed no turbidity after incubation. The turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. The minimum bactericidal concentration (MBC) was determined by sub culturing 50µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on sub culturing was taken as MBC.

Statistical Analysis

The basics statistics means and std deviation of the measured parameters were estimated. Data were statistically analysed using one way ANOVA test.

RESULTS

Table 1: Minimum Inhibitory Concentrations (MIC) of the Crude Extracts of four different parts form *B. nigra* against on five pathogenic organisms

Name of the solvents	Plant part	*S. aureus*	*P. vulgaris*	*P. mirabilis*	*E. aerogens*	*E. coli*
Pet. ether	Leaf	3.0±0.5	-	1.5±5.34	6.33±2.3	3.29±0.23
	Root	2.7±0.4	4.81±1.37	5.6±1.49	5.0±1.2	-
	Flower	-	-	-	-	0.78±0.01
	seed	10.42±2.3*	14.7±2.8*	17.8±4.02**	14.3±4.37*	1.69±1.07
Methanol	Leaf	8.6± 0.42	5.6±2.31	7.3±2.54	0.47±0.01	-
	Root	-	1.35±3.11	-	-	1.05±0.22
	Flower	4.31±1.7	-	-	9.33±1.56	-
	seed	17.90±2.1*	5.30±0.67	8.32±2.0	2.30±0.4	11.47±3.3**
Chloroform	Leaf	-	-	3.41±1.45	-	2.07±0.32
	Root	0.31±0.07	1.76±0.02	-	1.4±0.15	-
	Flower	-	-	-	-	-
	seed	1.7±0.56	0.96±0.02	4.0±0.61	15.71.4±0.27**	1.3±0.06

IZ = Inhibition zone in mm (mean value; including 6 mm diameter of disc), AI = Activity Index (IZ developed by extract/IZ developed by standard), ± = SEM, (–) = No activity

Antimicrobial activity (assessed in terms of inhibition zone and activity index) of the plant extracts, tested against selected microorganisms were recorded (Table 1). In the present study total three kinds of extracts of different parts of *B. nigra* were tested for their bioactivity, among the three extracts showed significant antimicrobial potential against tested microbes. However, two extracts showed no activity against any selected microorganism at tested concentration. Chloroform flower showed refusal activity against five tested bacteria. However, maximum zone of inhibition observed in seed extract with polar and non polar solvents such as Pet. Ether, Chloroform and methanol against' *S. aureus* (17.90±2.1), *P. mirabilis* (17.8±4.02)**,** *E. aerogens* **(**15.71.4±0.2), *P. vulgaris* (14.7±2.8)**,** *E. coli* **(**11.47±3.3). Comparison of the antimicrobial activities of the fractions with that of the crude extract indicated that the non-polar fractions in many cases are stronger in activity at the two concentration levels than the crude extract. The results are expected because 80% methanol, being highly polar, is unable to extract as much of the active compounds as can be extracted with non-polar solvents like petroleum ether and chloroform. The antimicrobial activity of the non-polar fractions of the two species showed similar activity profile on the selected strains to that of the crude extract, i.e. the petroleum ether and chloroform fractions of seeds were more active against the bacteria and that of leaf, stem and flower against the tested pathogens. Moreover, the present results depicted significant minimum inhibitory activity showed the seed pet. Ether extract against the four organisms such as *S. aureus, P. mirabilis, P. vulgaris* and *E. aerogens*. In addition, methanolic extract demonstrate s the significant activity on *S. aureus* and *E.coli* and chloroform extract revealed *E. aerogens* respectively.

DISCUSSION

Resistance in microorganisms to many antibiotics has resulted in morbidity and mortality from treatment failure and increased health care costs. Though a number of antibiotics are available but increasing capability of microbes to develop multidrug resistance has encouraged search for new, safe and effective bioactive agents of herbal origin. Most of the natural products are found to be more effective with least side effects as compared to commercial antibiotics so that reason they are used an alternated remedy for treatment of various infections. In addition, the activity of the petroleum ether fraction of at 25 mg/ml is almost equivalent to the activity of the crude extract at 100 mg/ml. This result supports the fact that the active compounds are more concentrated in this fraction. It also underlines the importance of testing activities of the different fractions before reporting that such type of herbal drugs are inactive by simply looking at the results of the crude extract, especially for those drugs having a long history of use by the local people. In general, results of antimicrobial activity tests of the fractions indicated that further studies carried out on the petroleum ether and chloroform fractions of these two species might lead to the isolation of the desired active compound(s) should be necessary.

Spices are defined as plant substances to enhance flavor, they include leaves (mint and coriander), flower (clover), bulbs (garlic, turmeric), fruits (black pepper), stem (cinnamon), rhizomes (ginger and turmeric) [11, 12]. Medicinal plants produce certain bioactive molecules which showed both antibacterial and antifungal activities[13]. Many medicinal plants produce antioxidant and antimicrobial properties which protect the host from cellular oxidation reactions and other pathogens highlighting the importance of search for natural antimicrobial drugs[14, 15]. This kind of similar view also been published by Essawi and Srour, (2000)[7] such as most of the foods borne bacterial pathogens are sensitive to extracts from plants such as garlic, mustard, onion and oregano. Gram positive bacteria are more sensitive to antimicrobial compounds in spices than G-negative

bacteria[8]. *B. nigra* L. (Asteraceae) was selected in the present study for evaluation of their antimicrobial activities. Both the plants are well adapted to the harsh (xerophytic) climatic conditions and are well known for their medicinal properties among local natives[16].

In Ayurvedic medicine *B. nigra* L is recorded as a hepatic stimulant and protectant. Decoctions from *C. decidua* root bark have been traditionally used for dropsy, anaemia, arthritis, and gout. It is used for the treatment of asthma, ulcer, piles, and urinary problems[17]. *Escherichia coli*, *Staphylococcus aureus, Proteus mirabilis*, and *Candida albicans* have been proved to be major causal organisms of various human infections and have been selected for the present study. *E. coli* and *P. mirabilis* are the culprits for human urinary tract infections[18, 3, 19] and most of the human intestinal infections are due to the bacterium *E. coli*. *S. aureus* causes a variety of supportive, wound infections and food poisoning in human beings[21, 22].

CONCLUSION

Comparison of the antimicrobial activities of the fractions with that of the crude extract indicated that the non-polar fractions in many cases are stronger in activity at the two concentration levels than the crude extract. These results are expected because 80% methanol, being highly polar, is unable to extract as much of the active compounds (which are non polar) as can be extracted with non-polar solvents like petroleum ether and chloroform. The antimicrobial activity of the non-polar fractions of the two species showed similar activity profile on the selected strains to that of the crude extract, i.e. the petroleum ether and chloroform fractions of seed and root of *B. nigra* were more active against the bacteria *P. mirabilis* and *S. aureus*. In addition highest zone of inhibition observed in seed extract with polar and non polar solvents such as Pet. Ether, Chloroform and methanol against' *S. aureus* (17.90±2.1), *P. mirabilis* (17.8±4.02)**,** *E. aerogens* **(**15.71.4±0.2), *P. vulgaris* (14.7±2.8)**,** *E. coli* **(**11.47±3.3). Comparison of the antimicrobial activities of the fractions with that of the crude extract indicated that the non-polar fractions in many cases are stronger in activity at the two concentration levels than the crude extract.

ACKNOWLEDGEMENT

We would like to sincerely thank Rev. Fr. Premkumar (M.S.W) Secretary and Correspondent in Malankara Catholic College, Mariagiri for valuable suggestions, insightful advices and a boost of the scientific endeavors.

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Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:

S. Sujatha and Akila Suresh. Polar and non polar solvent extraction and pharmacological evaluation of four different parts from Brassica nigra (Koch.) plant. *J Pharm Sci Innov*. 2013; 2(3): 27-29.