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ANTIMICROBIAL ACTIVITY OF FRUITS OF *SOLANUM NIGRUM* AND *SOLANUM XANTHOCARPUM*

KHIZAR ABBAS[1,2], UZMA NIAZ[1], TALIB HUSSAIN[1], M. ASIF SAEED[1], ZEESHAN JAVAID[3]*, ARFAT IDREES[4] and SHAHID RASOOL[5]

[1]University College of Pharmacy, University of the Punjab, Lahore, Pakistan
[2]College of Pharmacy, GC University, Faisalabad, Pakistan
[3]Akson College of Health Sciences, Mirpur University of Science & Technology, Mirpur, A.J. & Kashmir, Pakistan
[4]Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan
[5]Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan

Abstract: Current study was conducted to investigate antimicrobial activity of fruit extracts of two Solanaceous plants (*Solanum nigrum* and *Solanum xanthocarpum*) found in Pakistan. Petroleum ether, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water were utilized for extraction. The highest percentages of polar components of both the species were extracted by water; little amount of non-polar components by petroleum ether while very low quantities by other solvents. Antimicrobial activities were estimated by measuring zones of inhibition through hole-plate diffusion method, against three species of Gram positive bacteria, five species of Gram negative bacteria and three species of fungi selected for this study. Doses of 5, 10 and 15 mg/mL prepared through methanolic extracts of each plant's powdered fruit material displayed significant zones of inhibition against all three Gram positive bacteria, three of the Gram negative bacteria out of five and against all three fungi. Although these doses exhibited significant zones of inhibition but they are not as potent as standards: ampicillin or amphotericin B. The present study assures the possible potential of antimicrobial as well as antifungal activity of fruit extracts of these plants.

Keywords: antimicrobial activity, *Solanum nigrum*, *Solanum xanthocarpum*, zones of inhibition, antifungal activity, solvent extraction

Pakistan is among the reasonably diverse countries in biological resources. Here people trust on natural sources, have invaluable and uninterrupted practice of the use of medicinal plants and other natural resources for healthcare necessities (1). The knowledge of naturally occurring medicinal plants (drugs) was slowly acquired by humans on the basis of experience (2). Plants play important function in developing modern medicines as they contain active phytochemical components. They have beneficial effects on the community by improving the health of human beings by treating many diseases for many years (3, 4). For such activity, the source of phytochemicals have commonly found in leaves, barks, roots, flowers, fruits and seeds of the plants (5). Sandhu and Heinrich (6) and Gupta et al. (7) has pointed out that many rural communities in developing countries depend on plant sources for their nutrient and scrounge, making household utilities as well as utilized them for fire, shadow and as herbal drugs.

The plants usually possess antimicrobial substances for their own protection from microbial infection and deterioration; that's why they are being used for the conservation and safety of food products (8–10). Ushimaru et al. (11) assessed the antimicrobial activity of aqueous and ethanol extracts of nine Nigerian species against four nutrient borne bacteria for checking their pharmacological activity in the direction of formulating new anti-abscessed agents. Many analytical reports showed that *Solanum* plants are important source of large number of phytochemical compounds with substantial curative application against human pathogens (12). So they could be assessed as an alternate way to fight against bacterial diseases (13).

* Corresponding author: e-mail: drmianjavaid@hotmail.com; phone: 0092-300-7311771

Black nightshade *(Solanum nigrum* L.) is a weed of amentaceous land, gardens, and soils rich in nitrogen and is broadly distributed in Pakistan (14). The fruit of *S. nigrum* contains many ingredients including fatty acid, tannins, cellulose, resins, dextrin, ash and moisture. Methanolic root extracts of *S. nigrum* showed antimicrobial and antifungal properties (15). It was also demonstrated that the *S. nigrum* extract acts as a larvicidal agent against five laboratory colonized strains of mosquito species (16). Fruits also contain tropeine, an alkaloid having mydriatic action, along with solanine (17). Four anti-cancer steroidal glycosides; solasonine, solamargine, diosgenin and solasodine were isolated from the immature *S. nigrum* fruits (18). Recent phytochemical analysis of *S. nigrum* fruit has resulted in the isolation of two novel disaccharides along with protein, fibre, carbohydrate, minerals like magnesium, phosphorus and vitamin C, B and folic acid (19).

Solanum xanthocarpum is also recognized as Indian night-shade or yellow berried nightshade plant. It is well versed in India and Pakistan; often in wastage places, on roadsides and in open spaces as well. Its fruit contains carpesterol, glucose, galactose, potassium chloride, a number of steroidal compounds and alkaloids mainly in the form of glycoalkaloids. The flavanoids quercitrin and apigenin glycosides were the major chemical constituents present in the fruits of *S. xanthocarpum* (20, 21). Many therapeutic activities of the fruits of this plant have been reported. Its' being used for itching and fever, reduces adipose tissues as well as seminal ejaculation (22, 23). The aqueous and organic solvent extracts of different parts of the plant demonstrated that all the extracts had very strong biological inhibition effects (24). Methanolic extracts of *S. xanthocarpum* and *Datura metel* exhibited highly significant antifungal activity against different species of pathogenic Aspergilli (25). Okram et al. (26) also investigated the strong inhibitory effects on the radiated growth of *Aspergillus niger* and *Trichoderma viride*.

The main objective of this study was to take into account the important aspect of antibacterial potential of fruits of *Solanum nigrum* L. and *Solanum xanthocarpum* Schrad & Wendl. along with antifungal activity and the main reason for choosing the fruits of these plants among the diversity is the reality that local people often employ these two important fruits as folk medicines for various infections. It is thus essential to assess the medicinal plants scientifically for various complaints that were made against the traditional medicine in the past.

EXPERIMENTAL

Chemicals and reagents

Ampicillin pure powder (Glaxo-Smith-Kline, Pakistan), Amphotericin B pure powder (Fada Pharma, Argentina), Nutrient agar medium (Merck lot no. NA806), Sabouraud dextrose agar media (Merck lot no. 111674/249), double distilled deionized water. Petroleum ether, chloroform, methanol, $DMSO_4$, ethyl acetate, acetone, dichloromethane. All the solvents were of analytical grade.

Plant collection

Fruits of *Solanum nigrum* L. and *Solanum xanthocarpum* Schrad & Wendl. were collected from the rural areas of district Muzaffargarh, South Punjab-Pakistan. After authentication by Herbarium staff of Bahauddin Zakariya University Multan, Pakistan, the voucher specimens were submitted in the Herbarium of Pharmacognosy Department, University College of Pharmacy, University of the Punjab, Lahore, Pakistan, for further reference. The fruits were dried under shade for a period of one month to achieve complete dryness and then pulverized to obtain coarse and fine powders.

Solvent extraction

The weighed quantities of dried powdered materials collected from fruits of both the plants were extracted with various solvents successively such as petroleum ether, chloroform, dichloromethane, ethyl acetate, acetone and water. The maceration was carried out for seven days at room temperature for individual solvent and successively repeated for each solvent with a sequence as mentioned above. The extracts for each solvent were collected separately, then filtered and dried under vacuum in a rotary evaporator at $40 \pm 5^{o}C$. These dried extracts were weighed to calculate the total percentage yield for individual solvent and were redissolved in dimethyl sulfate ($DMSO_4$) for antimicrobial activity analysis and stored in labelled sterile screw capped bottles.

Preparation of methanolic extract

The weighed quantities of dried powdered materials of fruits of both the plants were extracted individually thrice with each time using 1 liter of methanol by maceration for seven days at room temperature. The methanolic extracts obtained from both the plants were filtered, dried and stored according to the procedure as mentioned above.

Test microorganisms

Antibacterial and antifungal studies were conducted upon three species of Gram positive bacteria; *Micrococcus varians* (ATCC No. 9341), *Micrococcus luteus* (ATCC No. 9342), *Staphylococcus aureus* (ATCC NO. 25923), five species of Gram negative bacteria; *Salmonella typhi* (ATCC No. 19430), *Pasteurella maltocida* (ATCC No. 51687), *Escherichia coli* (ATCC No. 25922), *Klebsiella pneumoniae* (ATCC NO. 700721), *Vibrio cholerae* (ATCC No. 39541) and three species of fungi; *Aspergillus niger* (ATCC No. 16404), *Aspergillus flavus* (ATCC No. 204304), *Aspergillus fumigatus* (ATCC No. 204305). Pure cultures of these microorganisms were collected from Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

Assay methodology

Hole-plate diffusion method was employed to test the antibacterial and antifungal activity of fruit extracts of both the plants. A suitable suspension (inoculum) of each microorganism was prepared by incorporating one loop full of fresh microorganism in 10 mL of sterilized water for injection near flame. Ten milliliters of prepared inoculum of each Gram positive and Gram negative microorganism was then separately poured in each of 500 mL of sterile liquefied nutrient agar medium for determination of antibacterial activity. Similarly, 10 mL of prepared inoculum of fungal species was separately poured in each of 500 mL of sterile liquefied Sabouraud dextrose agar medium for determination of antifungal activity. These media were properly labelled for each microorganism and then gently shaken to allow uniform mixing of the inoculum with the medium. After mixing, they were poured in labelled sterile Petri dishes in a specified quantity to maintain a depth of media up to 8 mm, while it was being spread with the help of spreader. Each prepared Petri dish was gently rotated for proper and uniform spreading of medium and allowed to solidify at room temperature.

For different solvent extracts of *S. nigrum*, eight holes were made in the solidified medium at uniform distance from each other with stainless steel borer and numbered as 1 to 8. Hole no. 1 was filled with positive control reference solution while hole 8 with negative control $DMSO_4$ solvent. Holes from 2 to 7 were filled with sample solutions of different solvents extract of *S. nigrum* including methanolic extract at a dose of 20 mg/mL.

For comparative antibacterial and antifungal activities of both the plants, three doses i.e., 5, 10 and 15 mg/mL of methanolic extracts of fruits of both the plants were used. For methanolic extract five holes were made in the solidified medium. The holes were numbered as 1, 2, 3, 4, and 5 and filled aseptically with reference and sample solutions. Reference or positive controlled antibacterial/antifungal solution were filled in hole no. 1, while hole no. 2, 3 and 4 were filled with methanolic extract of fruit of *S. nigrum* with 5, 10 and 15 mg/mL concentrations, respectively. The solvent extracts were diluted to solutions of different concentrations with $DMSO_4$ solvent. Hole no. 5 was filled aseptically with the negative controlled reference solvent sample i.e., $DMSO_4$. The different solvents and methanolic extracts of fruit of *S. xanthocarpum* were also treated in a similar manner.

For antibacterial activity, the Petri dishes were kept in an incubator at 32 ± 2.5^oC for 48 h, whereas for antifungal activity; the incubation temperature was 22 ± 2.5^oC for 72 h. In each case, zones of inhibition were observed and measured with the help of vernier calliper. The experiments were performed in three replicates. Results were expressed as the mean zones of inhibition.

Statistical analysis

The mean zones of inhibitions caused by the solvent extracts of the plant's fruit materials and standard drugs were calculated and reliability of the samples was assessed by calculating standard deviation.

RESULTS AND DISCUSSIONS

Fruits of *S. nigrum* and *S. xanthocarpum* collected from dissimilar localities in different seasons showed that the local mesophytic soil and climatic conditions affected the advent of both the plant fruits. A great variation in the appearance of fruits of both the plants was found in the size and shape arrangements and also in the number and color of the berries and seeds etc. On the basis of such ecological variations, different chemical identification tests were performed to determine the naturally occurring as well as secondary metabolites (alkaloids, flavonoids, sapogenins, steroids, sterols etc.). Positive results indicated the presence of these metabolities, which caused the fruits to adjust themselves according to different conditions (27).

The extracts obtained by extraction of dried powder of fruits of both the plants by using different types of solvents, were compared for efficiency of eluting solvents by calculating the percentage yield of extracted materials. It was found that maximum

Table 1. Percentage yield of the extracted materials of dried powdered fruits of *S. nigrum* and *S. xanthocarpum* plants by different solvents.

Solvents	Percentage yields	
	Solanum nigrum	*Solanum xanthocarpum*
Petroleum ether	5.79	6.68
Chloroform	8.47	9.42
Dichloromethane	10.26	11.73
Ethyl acetate	12.87	13.27
Acetone	14.89	16.29
Water	41.63	42.47
Total yield	94.07	99.86
Methanol	36.52	39.56

Table 2. Antimicrobial activity of various solvent extracted materials of dried powdered fruit of *Solanum nigrum* (dose level = 20 mg/mL).

Microorganisms	**Zones of inhibition (mm) ± SE**						
	P-ether	**Chlo.**	**Dimet.**	**Et-act.**	**Acet.**	**Meth.**	**Water**
Gram positive bacteria							
Micrococcus luteus	3.2 ± 3.1	9.2 ± 1.25	8.5 ± 2.0	6.5 ± 3.2	7.6 ± 1.3	3.4 ± 2.1	13.5 ± 3.4
Staphylococcus aureus	3.4 ± 2.2	8.5 ± 2.12	7.5 ± 1.8	7.2 ± 2.0	8.3 ± 2.0	15.2 ± 3.5	14.7 ± 2.4
Gram negative bacteria							
Salmonella typhi	4.5 ± 2.4	7.6 ±1.8	7.6 ± 1.5	6.7 ± 2.5	6.5 ± 2.0	15.2 ± 3.3	15.3 ± 3.2
Escherichia coli	6.2 ± 1.3	6.7±1.4	8.5 ± 2.0	7.5 ± 2.3	7.9 ± 2.1	14.3 ± 2.6	16.4 ± 3.0
Fungi							
Candida albicans	3.7 ± 2.0	5.8 ± 2.6	6.4 ± 3.3	6.7 ± 1.3	5.3 ± 1.0	7.6 ± 1.0	4.8 ± 2.10

P-ether = Petroleum ether; Chlo. = Chloroform; Dimet. = Dichloromethane; Et-act. = Ethyl acetate; Acet. = Acetone; Meth. = Methanol

Table 3. Antimicrobial activity of various solvent extracted materials of dried powdered fruit of *Solanum xanthocarpum* (dose level = 20 mg/mL).

Microorganisms	**Zones of inhibition (mm) ± SE**						
	P-ether	**Chlo.**	**Dimet.**	**Et-act.**	**Acet.**	**Meth.**	**Water**
Gram positive bacteria							
Micrococcus luteus	3.6 ± 2.5	7.5 ± 2.2	9.7 ± 1.2	9.8 ± 2.1	6.2 ± 2.3	13.7 ± 0.4	12.7 ± 2.0
Staphylococcus aureus	3.9 ± 3.2	8.3 ± 3.1	8.8 ± 2.4	7.4 ± 3.2	8.0 ± 2.1	14.1 ± 2.4	14.0 ± 2.2
Gram negative bacteria							
Salmonella typhi	6.5 ± 2.2	6.6 ± 2.4	8.9 ± 2.6	5.7 ± 3.1	6.0 ± 1.1	14.1 ± 1.3	15.7 ± 1.2
Escherichia coli	8.6 ± 1.	4 7.6 ± 1.5	9.7 ± 2.3	8.1 ± 2.5	8.9 ± 0.7	14.2 ± 2.4	17.8 ± 2.4
Fungi							
Candida albicans	5.6 ± 2.3	7.7 ± 1.8	7.3 ± 3.7	6.0 ± 1.0	4.3 ± 1.2	7.0 ± 0.5	7.9 ± 2.6

P-ether = Petroleum ether; Chlo. = Chloroform; Dimet. = Dichloromethane; Et-act. = Ethyl acetate; Acet. = Acetone; Meth. = Methanol

Table 4. Comparative antimicrobial activities of the methanolic extract of the dried powdered fruit of *S. nigrum*, *S. xanthocarpum* and standard drugs.

Microorganisms	**Zones of inhibition (mm) ± SE**						
	***S. nigrum* (Dose = mg/mL)**			***S. xanthocarpum* (Dose = mg/mL)**			**Stand. drug***
	5	**10**	**15**	**5**	**10**	**15**	**(1 or 2)**
Gram positive bacteria							
Micrococcus varians	10.2 ± 1.3	12.2 ± 1.0	13.3 ± 1.0	7.4 ± 2.3	9.6 ± 2.1	10 ± 3.2	14 ± 5.2
Micrococcus luteus	10.1 ± 2.0	11.8 ± 1.0	12.5 ± 2.3	8.6 ± 3.2	9.2 ± 2.3	11 ± 2.1	31 ± 4.3
Staphylococcus aureus	9.1 ± 3.1	10.3 ± 2.1	11.3 ± 3.2	07 ± 4.2	9.6 ± 3.4	11.3 ± 2.0	9 ± 2.5
Gram negative bacteria							
Salmonella typhi	11.3 ± 3.1	12.4 ± 4.0	14.6 ± 2.0	10.6 ± 1.2	12.3 ± 1.2	13 ± 2.3	25 ± 2.4
Pasteurella maltocida	8.2 ± 1.4	10.6 ± 2.1	10.7 ± 1.3	12.5 ± 1.3	14.2 ± 1.0	14.9 ± 3.3	20 ± 2.2
Escherichia coli	9.0 ± 2.1	11.0 ± 1.1	11.6 ± 2.1	12.0 ± 1.0	13.0 ± 1.0	14.0 ± 1.1	15 ± 2.3
Klebsiella pneumoniae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	23 ± 3.6
Vibrio cholerae	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	34 ± 3.2
Fungi							
Aspergillus niger	4.6 ± 1.5	6.7 ± 2.1	9.3 ± 3.1	5.9 ± 1.0	6.4 ± 2.3	6.5 ± 2.4	11 ± 3.1
Aspergillus flavus	5.5 ± 1.3	6.6 ± 3.2	6.9 ± 2.0	7.5 ± 2.7	8.5 ± 2.5	9.3 ± 1.5	12 ± 2.5
Aspergillus fumigatus	6.6 ± 3.2	7.1 ± 1.0	7.6 ± 3.2	7.4 ± 1.4	7.8 ± 2.4	8.3 ± 2.3	13 ± 3.4

*Stand. drug – ampicillin in dose 125 mg/mL (**1**) and amphotericin B in dose 5 mg/mL (**2**).

efficiency was shown by water for both the fruits i.e., 41.63 and 42.47%, and methanol 36.52 and 39.56% for *S. nigrum* and *S. xanthocarpum*, respectively, whereas the least amount was extracted by petroleum ether as shown in Table 1. The results elaborate that polar solvents like methanol and water yielded a higher percentage of extracted materials mainly of polar components as compared to other solvent extracts (28). Among these two solvent extracted materials, water was found to be the most potential candidate, which extracted the highest percentage of highly polar components present in the powders of both the species (29). On the other hand, petroleum ether is non-polar in nature and it extracted the non-polar materials but yielded in least quantity, which is one of the evidence that these plants contain very small amount of non-polar compounds. Chloroform, dichloromethane, ethyl acetate and acetone possess intermediate polarities, so they extracted the components from fruits that possess intermediate polarities but extracted in minute quantities that is also indicative of very low percentage of these components in the extracts (30).

The results of preliminary antimicrobial activities of various solvent extracts of fruits of both the plants against three types of microorganisms (Gram positive bacteria, Gram negative bacteria and fungi) have been demonstrated in Tables 2 and 3. When these results were critically evaluated, it was found that the extracts, which have been extracted by using more polar solvents have greater potential for antimicrobial activity as compared to those extracted with less polar solvents. The antimicrobial substances generally possess intermediate polarity and smaller molecular weight antimicrobial substances are generally miscible with polar solvents, so can be easily concentrated in the polar solvent extracts. Also the antimicrobial activity is always dependent on the concentration of extracted antimicrobial metabolites, which can be enhanced with the increased polarity of solvents (31).

The observation that the compounds having activity against microorganisms are more soluble in polar solvents provides the basis for comparative study of extracts of fruits of plants to find the minimum inhibitory concentration against these organisms when these were extracted with a polar solvent – methanol. Findings of comparative antimicrobial activities of three doses i.e., 5, 10 and 15 mg/mL of methanolic extracts of fruits of *Solanum nigrum* and

Solanum xanthocarpum, along with the standard drugs (positive controlled reference drugs i.e., ampicillin or amphotericin B) against three species of Gram positive bacteria, five species of Gram negative bacteria and three species of fungi depict significant zones of inhibition against three Gram positive bacteria (*Micrococcus varians*, *Micrococcus luteus* and *Staphylococcus aureus*), three Gram negative bacteria (*Salmonella typhi*, *Pasteurella maltocida* and *Escherichia coli*) out of five and three species of fungi (*Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*). Although three doses of both the extracts displayed well marked inhibitory effects and exhibited significant zones of inhibition against nine microorganisms, yet it appeared to possess lesser activities than the standard ampicillin (with standard dose = 125 mg/mL), and amphotericin B (with standard dose = 5 mg/mL). So it is clear here that these extracts do possess antimicrobial and antifungal activity but not as potent as the standard antibacterials and antifungals (32). Therefore, we would like to state that the constituents of fruit extracts may serve as a beneficial source of industrial drugs useful in treatment of some bacterial infections (33). Since both the plants contain alkaloids, glycosides, lignins, tannins, and terpenoid compounds like monoterpenes, sesquiterpenes, diterpenes or triterpenes, probably these compounds get through the bacterial and fungal cell wall/membrane and supress their growth or if these compounds deeply penetrated, might kill them completely. These results are more or less similar to the previous findings by other workers, who explored the antibacterial and antifungal potential of natural products, against wide ranges of microorganisms, particularly from various members of family Solanaceae (34, 35).

From comparative study, it was found that the fruit extract of *S. xanthocarpum* has more antimicrobial activity against Gram negative bacteria as well as showed greater antifungal activity as compared to *S. nigrum,* whereas *S. nigrum* was found to be more potent against Gram positive bacteria as compred to *S. xanthocarpum*. The reason for being *S. xanthocarpum* more potent is that it contains specifically carpesterol and similar other steroidal glycoside, which are absent or not present in appreciable or distinguishable quantities in *S. nigrum* (36). These results have been shown in Table 4.

CONCLUSION

Above mentioned results revealed that by increasing the polarity of various solvents, concentration of extracted compounds and their antibacterial as well as antifungal activity increases. That's why the rural community mostly use it as a folk medicine. This investigation has created the possibility of use of these plants in drug development for human consumption. However, the effects of these plants on more pathogenic organisms and toxicological investigations need to be carried out.

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