Antagonistic activity of *Solanum nigrum* (L.) extracts against causative organisms of diarrhoeal diseases

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Abstract: This study screened the ethanol extracts of *Solanum nigrum* (Solanaceae) against causative organisms of diarrhoeal diseases to ascertain its effectiveness in the treatment of the diseases. The leaf of *S. nigrum* was screened for the presence of secondary metabolites. The antibacterial assay of the extracts against indicator organisms (*Escherichia coli* ATCC 25922; *Escherichia coli* 0157:H7; *Salmonella typhimurium* ATCC 13311; *Salmonella typhi*; *Shigella flexneri* KZN; *Vibrio cholerae*) was done using agar-well diffusion method. The Minimum Inhibitory Concentration at 0.1, 0.5, 1.0 and 5.0 mg/ml of various extracts against indicator organisms (10^2 cfu/ml) was determined using agar dilution method. The plant contained alkaloids, flavonoids, phenols, saponins and tannins. The ethanol extract of the berry was most active against *Escherichia coli* ATCC 25922 with 20.0 mm zone of inhibition. The root extract was active against *Escherichia coli* 0157:H7 (30.0 mm), *Shigella flexneri* KZN (20.0 mm) and *Vibrio cholerae* (35.0 mm). Overall, the root extract gave the highest antibacterial activity against 4 out of 6 indicator organisms. The leaf extract of *S. nigrum* gave the least (0.1 mg/ml) MIC value against all organisms. From this study, the extracts of *S. nigrum* showed the potential to be used in treatment of diarrhoeal diseases.
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1. Introduction

Solanum nigrum is a wild vegetable with many therapeutic values in folk medicine worldwide. The roots are used to treat stomach-ache. The leaves have antiseptic and antimicrobial properties; the leaves are used for the treatment of ulcers, wounds, and inflammation as well as skin diseases (Jansen, 2008). The literature is full of information on the pharmacological activities of *S. nigrum* but there is dearth of report on its antidiarrhoeal activity. The fruit extract (250mg/kg) of *S. nigrum* showed significant antioxidant and antihyperlipidemia against ethanol-induced toxicity in rats (Arulmozhi et al., 2010). It is used in the management of gastric ulcers in ayurveda. *S. nigrum* fruits extract significantly inhibited the gastric lesions induced by cold restraint stress (76.6%), indomethacin (73.8%), pyloric ligation (80.1%); ethanol (70.6%) being more potent than omeprazole in experimental ulcer models (Jainu and Devi, 2006). The powder and methanol extract of the aerial parts of *S. nigrum* significantly decreased the ulcer index in rats (Akhtar and Munir, 1989). Lin et al. (2007) reported its anticancer effect, 2mg/ml and 5mg/ml of *S. nigrum* extract induced cell death in HepG2 cells. The extract induced cell death in hepatoma cells through two distinct antineoplastic activities, the ability to induce apoptosis and autophagocytosis, therefore, indicating that it may be useful in the treatment of liver cancer. Of relevance is the reported therapeutic value of *S. nigrum* in the management of Inflammatory Bowel Disease (IBD) in traditional Iranian medicine (Rahimi et al., 2010).

Diarrhoeal diseases constitute enormous public health problem worldwide, especially in developing countries where the diseases form the main cause of child mortality, particularly in children younger than 5 years of age. Global deaths from diarrhoea of children (< 5 years) were estimated at 1.87 million, approximately 19% of total child deaths. African and South-East Asia regions combined have 78% (1.46 million) of all diarrhoea deaths occurring among children in the developing world; 73% of diarrhoea associated deaths are concentrated in just 15 developing countries (Boschi-Pinto et al., 2008). The excessive death rate resulting from diarrhoea outbreak had led to the search for medicinal plants with antidiarrhoeal activity, which could be economically handled by poor people in developing countries (Lewu & Afolayan 2009).

In view of scarcity of information on the antidiarrhoeal activity of *S. nigrum*, this study screened the ethanol extracts of berry, leaf, stem and root of *S. nigrum* against causative organisms of diarrhoea: *Escherichia coli*, *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Salmonella typhi*, *Shigella flexneri* and *Vibrio cholerae*. This was with the aim of ascertaining the potential of the plant in the treatment of diarrhoeal diseases.

2. Materials and Methods

Fresh and healthy whole plants of *S. nigrum* were collected from University of Fort Hare (UFH) Research Farm, Alice Campus, Eastern Cape Province of South Africa. The plants were harvested at maturity in December, 2012. *S. nigrum* was identified at Griffin's Herbarium of the Department of Botany, University of Fort Hare, Alice, South Africa. A voucher specimen (BVE11/017) of the plant was prepared and deposited at the Herbarium.

Whole plants of *S. nigrum* were separated into berries, leaves, stems and roots. The different plant parts were washed; oven dried (30^{o}C) and powdered. 200g of each sample was extracted in 800 ml of ethanol (90%) by shaking for 24 h. The extracts were filtered, concentrated and dried at room temperature. The extracts were kept in the refrigerator (4^{o}C) for further use. The powdered leaf was screened for the presence of secondary metabolites or natural products. The steps taken are in line with standard procedures (Harborne, 1984; Sofowora, 1993; Evans, 1996).

Bacterial isolates were type strains and Laboratory isolates: *Escherichia coli* (ATCC 25922); *Escherichia coli* 0157:H7 (Laboratory isolate); *Salmonella typhimurium* (ATCC 13311); *Salmonella typhi* (Laboratory isolate); *Shigella flexneri* (KZN); *Vibrio cholerae* (Laboratory isolate). The type strains were procured from the South African Bureau of Standards (SABS) and Laboratory isolates were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. All bacteria were cultured and maintained on nutrient agar (Biolab) throughout the experiment.

The extract (10g) of each sample was dissolved in 100 ml of ethanol to give an extract concentration of 100 mg/ml that was used for the antibacterial test. All overnight cultures of organisms were grown in nutrient broth (Biolab) at 37^{o}C. 19 ml of sterile nutrient agar was inoculated with 1 ml of the broth (10^2 cfu/ml) and poured into Petri dishes. The agar was allowed to gel and 2 wells were made in the agar with a 4 mm cork borer. Into these wells 80µl of either extract or ethanol (control) were poured. All plates were incubated at 37^{o}C for 24 h and the zones of inhibition were recorded (Hood et al., 2003).

The extracts were prepared in four different concentrations: 0.1; 1.0; 0.5 and 5.0 mg/ml for the Minimum Inhibitory Concentration (MIC) test. 1 ml of each extract concentration was added to 9 ml of sterile nutrient agar, poured into Petri dishes and allowed to set. The bacteria were cultured in nutrient broth overnight at 37^{o}C; each broth culture was diluted with fresh sterile broth . The organisms (10^6 cfu/ml) from the freshly inoculated nutrient broth were streaked in radial patterns on the agar plates (Meyer and Afolayan, 1995). The extraction solvent (ethanol) was used as control. Plates were incubated at 37^{o}C for 24 h. Total suppression of growth of the organism was required for an extract to be declared active at a particular concentration.

3. Results and Discussions

The leaf of *S. nigrum* contained alkaloids, flavonoids, phenols, saponins and tannins (Table 1). The extract from the berry was the most active against *E. coli* with 20.0 mm zone of inhibition (Fig. 1); followed by the root extract (16.0 mm) and the stem extract (10.0 mm) gave the least inhibition. The root extract exhibited the highest inhibitory activity against *E. coli* 0157:H7 with 30.0 mm zone of inhibition, followed by the stem extract (20.0 mm) and the berry extract (15.0 mm) was the least active. *S. typhimurium* was susceptible to the stem and root extracts with 15.0 mm zone of inhibition. The highest antagonistic activity against *S. typhi* was from the root extract (20.0 mm) and the leaf extract gave the least (14.0 mm). *S. flexneri* was most susceptible to the root extract (20.0 mm); followed by the berry extract (18.0 mm) and the least activity (15.0 mm zone of inhibition) was recorded for extracts of the leaf and stem. The root extract was significantly active against *V. cholerae* with 35.0 mm zone of inhibition, followed by the berry extract (30.0 mm) and the least activity of 20.0 mm was from the leaf and stem extracts. Overall, the root extract exhibited the highest spectrum of activity against 4 out of 6 bacterial isolates and was the most active part of the plant.

The MIC of extracts of the berry, leaf, stem and root of *S. nigrum* against bacterial isolates are presented in Table 2. The MIC of the extract from the berry was 0.1 mg/ml against *E. coli* 0157:H7, *S. typhimurium* and *V. cholerae*. It hindered the growth of *E. coli*, *S. typhi* and *S. flexneri* at 5.0 mg/ml extract concentration. The leaf extract significantly inhibited the growth of all isolates at the least extract concentration of 0.1 mg/ml. The stem extract exhibited high antibacterial activity against *E. coli* 0157:H7, *S. typhimurium* and *V. cholerae* with MIC value of 0.1 mg/ml. The stem extract gave a MIC value of 0.5 mg/ml against *E. coli*, *S. typhi* and *S. flexneri*. The root extract demonstrated high activity against *S. typhimurium*, *S. typhi*, *S. flexneri* and *V. cholerae* at the lowest (0.1 mg/ml) concentration of extract. Generally, the leaf extract recorded the lowest MIC value against all test organisms.

The results of the present study are in line with previous reports on the antibacterial activity of *S. nigrum*. Sridhar et al. (2011) reported that six different solvent extracts from the leaf, seed and root of *S. nigrum* showed antibacterial activity against *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *E. coli*, *Proteus vulgaris* and

Pseudomonas putida. The ethyl acetate seed extract showed strong activity against *Pseudomonas*, *P. vulgaris*, *Klebsiella* spp. and gave the lowest MIC values (1.50 - 4.50 µg/ml) against all the bacterial isolates tested. Its methanol and water leaf extracts exhibited antibacterial activity against organisms associated with opportunistic infections in diarrhoea viz. *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. The methanol extract gave highest antibacterial activity against test organisms compared to the water extract (Kavishankar et al., 2011). Also, the ethanol extract of the dried fruit of *S. nigrum* at the dose of 250mg/kg and 500mg/kg body weight, showed a significant ($P<0.01$ and $P<0.001$) antidiarrhoeal activity against castor oil induced diarrhoea in mice. It decreased the frequency of defecation and increased the mean latent period (Karmakar et al., 2010).

Table 1. Phytochemical components of leaf of *S. nigrum*

Phytochemical	Leaf
Alkaloids	+
Flavonoids	+
Phenols	+
Saponins	+
Tannins	+

Legend: + = Present

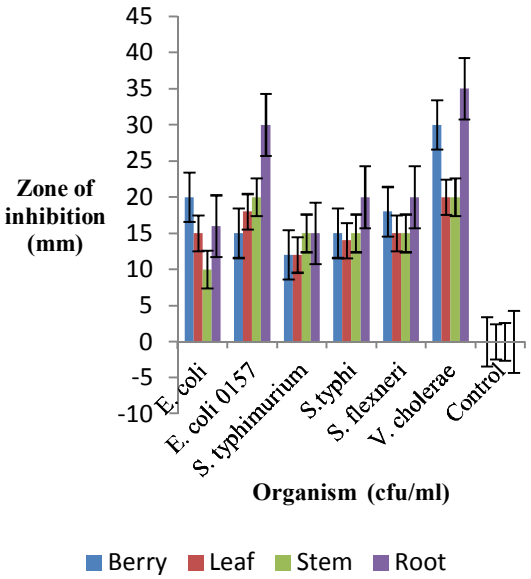

Figure 1. Antibacterial activity of ethanol extracts of *Solanum nigrum* against causative organisms of diarrhoeal diseases

Table 2. MIC of different parts of *Solanum nigrum* against pathogenic bacteria associated with diarrhoeal diseases

S/N	Organism	MIC (mg/ml)[a]			
		Berry	Leaf	Stem	Root
1.	*Escherichia coli*	0.5	0.1[b]	0.5	5.0
2.	*Escherichia coli 0157:H7*	0.1	0.1	0.1	0.5
3.	*Salmonella typhimurium*	0.1	0.1	0.1	0.1
4.	*Salmonella typhi*	0.5	0.1	0.5	0.1
5.	*Shigella flexneri*	0.5	0.1	0.5	0.1
6.	*Vibrio cholerae*	1.0	0.1	0.1	0.1

Legend: [a]Minimum inhibitory concentration.
[b]Lowest concentration of extracts tested

4. Conclusion

The ethanol extracts of the berry, leaf, stem and root of *S. nigrum* showed significant antibacterial activity against six pathogenic bacteria associated with diarrhoeal diseases viz. *Escherichia coli*, *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Salmonella typhi*, *Shigella flexneri* and *Vibrio cholerae*. The root extract was most active against test organisms. The observed antibacterial activity of the extracts could be attributed to the phytochemical components of *S. nigrum*. This study confirms the potency of the plant in the treatment of diarrhoeal diseases and justifies its use in traditional medicine as antidiarrhoeal remedy. Decoction, infusion or powder prepared from the roots of *S. nigrum* could serve as an effective herbal remedy in diarrhoeal diseases.

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References

1. Jansen PCM. *Solanum nigrum* L. [Internet] Record from Protabase. Schmelzer, G.H. & Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. < http://database.prota.org/search.htm>. Record display. *Solanum nigrum* L. 2008. Available at: http://www.prota4u.org/protav8.asp?h=M4&t=Solanum,nigrum&p=Solanum+nigrum#Synonyms. Accessed on 30 April 2013.
2. Arulmozhi V, Krishnaveni M, Karthishwaran K, Dhamodharan G, Mirunalini S. Antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract on the experimental model against chronic ethanol toxicity. Pharmacogn Mag. 2010: 6(21): 42-50.
3. Jainu M, Devi CS. Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: possible mechanism for the inhibition of acid formation. J Ethnopharmacol. 2006: 104: 156-63.
4. Akhtar, M. S. and Munir M. Evaluation of the gastric antiulcerogenic effects of *Solanum nigrum*, Brassica oleracea and Ocimum basilicum in rats. J Ethnopharmacol. 1989: 27: 163-76.
5. Lin HM, Tseng HC, Wang CJ, Chyau CC, Liao KK. Peng PL, Chou FP. Induction of autophagy and apoptosis by the extract of *Solanum nigrum* Linn in HepG2 cells. J Agric Food Chem. 2007: 55(9): 3620-28.
6. Rahimi R, Shams-Ardekani MR, Abdollahi M. A review of the efficacy of traditional Iranian medicine for inflammatory bowel disease. World J Gastroenterol. 2010: 16(36): 4504-14.
7. Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. Bulletin of the World Health Organization 2008: 86: 710–17.
8. Lewu FB, Afolayan AJ. Ethnomedicine in South Africa: The role of weedy species. Afr J Biotechnol. 2009: 8(6): 929-34.
9. Harborne JB.. Phytochemical methods. Chapman and Hall, London. 1984.
10. Sofowora, A. Medicinal plants and traditional medicine in African. Spectrum Books Ltd., Ibadan. 1993.
11. Evans WC. Trease and Evans' Pharmacognosy. W.B. Saunders Co. Ltd., Singapore. 1996.
12. Hood JR, Wilkinson JM. Cavanagh HMA. Evaluation of common antibacterial screening methods utilized in essential oil research. J Ess Oil Res. 2003: 15(6): 428-33.
13. Meyer JJM, Afolayan AJ. Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). J Ethnopharmacol. 1995: 47: 109-11.
14. Sridhar TM, Josthna P, Naidu CV. *In vitro* antibacterial activity and phytochemical analysis of *Solanum nigrum* (Linn.) - An important antiulcer medicinal plant. J Exp Sci. 2011: 2(8): 24-9.
15. Kavishankar GB, Lakshmidevi N, Mahadeva MS. Phytochemical analysis and antimicrobial properties of selected medicinal plants against bacteria associated with diabetic patients. Int. J Pharm and Bio Sci. 2011: 2(4): 509-18.
16. Karmakar UK, Tarafder UK, Sadhu SK, Biswas NN, Shill MC. Biological investigations of dried fruit of *Solanum nigrum* Linn. S. J. Pharm. Sci. 2010: 3(1): 38-45.

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