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Efficacy evaluation of extracts of *Brassica juncea* (Brown mustard) seeds as potential antimicrobial agent against pathogenic microbes

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Abstract
The World Health Organization (WHO) reported that about 80% of the world's population depends primarily on traditional medicine that mainly involves the use of plant extracts. The number of emerging multidrug resistant microbial strains is continuously increasing and has become one of the most serious threats to successful treatment of infectious diseases. In this study, antimicrobial potentials of the extracts of methanol, ethanol and ethyl acetate of *Brassica juncea* seeds were tested against different bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *and Salmonella typhi*) and fungi (*Aspergillus Niger*, *Mucor mucaralis*, *Tricophyton tonsurance*, *Microsporum ferrogenium and Aspergillus flavus*) strains by agar well diffusion method. The extracts showed a broad spectrum of antimicrobial activities, inhibition zones of bacteria strains ranged from 5 to 12mm for *Staphylococcus aureus*, 4 to 14mm for *Bacillus cereus*, 6 to 9mm for *Escherichia coli*, 5 to 11mm for *Pseudomonas aeruginosa* and 10 to 16mm for *Salmonella typhi* and fungi strains ranged from 5 to 10mm (*Aspergillus niger*), 9 to 14mm (*Mucor mucaralis*), 5 to 7mm (*Tricophyton tonsurance*), 12 to 13mm (*Microsporum ferrogenium*) and 10 to 16mm (*Aspergillus flavus*). The result of the study supports the immense medicinal properties of *Brassica juncea* which has revealed a significant scope to develop a novel broad spectrum of antimicrobial herbal formulation.

Keywords: *Brassica juncea*, antimicrobial agent, pathogenic microbes, herbal medicine

Introduction
Recently there has been a renewed interest in improving health and fitness through the use of more natural products. Herbs and spices have been used for thousands of years to enhance the flavor, color and aroma of food, also known for their preservative and medicinal value. The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. A large number of plants indeed were used to combat different diseases and known to possess antimicrobial activity [1].
Mustard has been used as medicine over hundreds of years which informed the choice for this study. Mustard seed is widely utilized in the preparation of varieties of edible sauces, pastes and pickle. The number of emerging multidrug resistant microbial strains is continuously increasing and has become one of the most serious threats to successful treatment of infectious diseases [2]. This increase is mainly attributed to indiscriminate use of broad spectrum antibiotic [3, 4]. *Brassica junncea* (Brown mustard) is one of the most popular species of mustard of the family cruciferae. The world health organization (WHO) reported that at about 80% of the world's population depends primarily on traditional medicine that mainly involves the use of plant extract [5].
This study therefore evaluated the efficacy of *Brassica juncea* (brown Mustard) seeds as potential antimicrobial agent against pathogenic microbes.

Materials and Methods
Sample collection
The seeds of *Brassica juncea* (brown mustard) were bought from a local market in Delta State, Nigeria.

Mustard Seed Extraction

The extraction reagents were methanol, ethanol, ethyl acetate, and dimethyl sulphoxide / acetone nitrile. About 10g of the mustard seed was placed in a beaker and 25ml of methanol added and mixed by vortexing. It was centrifuged at 3000rmp for 10 minutes. The supernatant was collected and transferred to a stoppered test tube by filtration. The resulting supernatant was evaporated to dryness with a gentle stream of nitrogen and reconstituted in 10ml dimethyl sulphoxide and was mixed by vortexing. The same procedure was repeated for that of ethanol and ethyl acetate.

Preparation of Dried Filter Paper Discs

Whatman filter paper no. 102 was used to prepare discs. Approximately 5mm in diameter was perforated using a perforator. These were placed in a petri dish after sterilization in autoclave.

Mustard Seed Extract Disc Placement

Mustard seed disc containing 3ml (3µl) concentration, as well as mustard seed were made using filter paper and then placed on the plates using sterile forcep. One sterile antibiotic disc was placed on the surface of an agar plate using a forcep. The forcep was sterilized by immersing in alcohol each time before placing another antibiotic disc. The disc was then gently pressed with the forcep to ensure complete contact with the agar surface and placed away from the edge of the plates so that it is easily measured. Once all discs were in place, the plates were inverted, and placed in a 37°C incubator for 24 hours.

Bacteria/ fungi suspension preparation

Media used: Nutrient agar, buffered peptone water, shigella salmonella agar, macconky agar, bacillus agar, and cetrimide agar. These media were prepared according to manufacturer's instruction. Using a sterile inoculating loop and needle for bacteria and fungi respectively, through aseptic techniques the test organisms of each colony was taken from the subculture plate. The organism was suspended in 4ml of normal saline and vortexed for overall suspension. Mcfarland standard solution was used as a reference to adjust the turbidity of individual bacterium isolate in the suspension (1×10^8). And 10 fold serial dilutions was made and plated for the antimicrobial sensitivity test.

Inoculation of Isolates on the Nutrient Agar Plate Proper

A sterile swab stick was dipped into the bacterial/ fungi suspension and the test organisms were suspended in 4ml of buffered peptone water. The swab was rotated against the side of the tube using firm pressure to remove excess fluid, but the swab was not dipped wet. The dried surface of the nutrient agar plate was inoculated by streaking the swab over the entire agar surface by rotating the plate at 60 degrees each time to ensure an even distribution of the inoculum.

Results

Table 1: Biochemical Characteristics of Isolates used for the test

S/N	Test Organisms	Biochemical Test										
		Gram Rxn	indole	MR	VP	citrate	Triple sugar Iron		CO_2	H_2S	Catalase	oxidase
							Slant	Butt				
1	*Pseudomonas aeruginosa*	-ve	-ve	-ve	-ve	+ve	A	A	-ve	-ve	+ve	-ve
2	*Bacillus cereus*	+ve	-ve	-ve	-ve	-ve	A	A	-ve	-ve	+ve	+ve
3	*Salmonella typhi*	-ve	-ve	+ve	-ve	-ve	-ve	A	-ve	+ve	+ve	-ve
4	*Escherichia coli*	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve
5	*Staphylococcus aureus*	+ve	-ve	+ve	+ve	+ve	A	A	-ve	-ve	+ve	-ve

***Note**: +ve= Positive; -ve= Negative; A= Absent; R×n= Reaction; MR= Methyl red; VP= Voges Proskauer; H_2S= Hydrogen Sulfide; CO_2 = Carbon dioxide

Table 2: Antibacterial Activity of Mustard Seed (Zone of Inhibitions in mm) Mean ± SD

S/N	Test organisms	Extracts		
		Ethanol	Methanol	Ethyl acetate
1	*Pseudomonas aeruginosa*	10±0.82	11±0.62	5±0.82
2	*Bacillus cereus*	12±0.47	14±0.48	4±0.85
3	*Salmonella typhi*	14±0.71	16±0.69	10±0.70
4	*E.coli*	8±0.41	9±0.77	6±0.65
5	*Staphylococcus aureus*	9±0.50	5±0.86	12±0.90

Table 3: Antifungal Activity of Mustard Seed (Zone of Inhibitions in mm) Mean ± SD

S/N	Test organisms	Extracts		
		Ethanol	Methanol	Ethyl acetate
1	*Aspergillus niger*	5±0.23	10±0.67	7±0.47
2	*Mucor mucaralis*	9±0.86	11±0.55	14±0.59
3	*Tricophyton tonsurance*	4±0.45	5±0.50	7±0.33
4	*Microsporum ferrogenium*	12±0.66	13±0.53	12±0.26
5	*Aspergillus flavus*	15±0.46	10±0.85	16±0.18

Table 4: Cultural Characterization and Identification of Fungi Isolates

S/N	Test Isolates	Pigmentation/ Culture Characterization
1	*Aspergillus niger*	White, dull yellow (reverse) raised
2	*Mucor mucaralis*	Whitish grey, white (reverse)
3	*Tricophyton tonsurance*	Grey brown, dark brown (reverse)
4	*Microsporum ferrogenium*	Creamy to buff coloured surface (no reverse)
5	*Aspergillus flavus*	Green, cream (no reverse), curled and raised

Discussion

In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly applied in the treatment of microbial infections [6, 7]. Research over the years has shown that medicinal plants possess in them bioactive components with valuable therapeutic relevance which can be harnessed in alternative medicine for the prevention, treatment and control of antibiotics resistant human pathogens as well as fungi [8]. This is because they have little or no toxicity challenge and can be easily obtained from our wild forest reserves. This study concentrated on fungi and both

Gram positive and negative bacteria strains. In the present work, the antibiotic potential of the ethanolic extracts of *Brassica juncea* showed a higher zone of inhibition in *Salmonella typhi* (14mm), *Baccilus cereus* (12mm) and *Pseudomonas aeruginosa* (10mm) but lowest on *Escherichia coli* (8mm). The results obtained from this study are in agreement with reports of Sunita *et al*. (2017) [9] and Malik *et al*. (2013) [10].

For methanol extract, the zone of inhibition was highest in *Salmonella typhi* (16mm), *Bacillus cereus* (14mm) and *Pseudomonas aeruginosa* (11mm) but lowest in *Staphylococcus aureus* (5mm). The zone of inhibition for ethyl acetate extract was highest in *Staphylococcus aureus* (12mm), *Salmonella typhi* (10mm) and *Escherichia coli* (6mm) but lowest in *Baccilus cereus* (4mm). The extracts were active against *Salmonella typhi which* is the cause of gastroenteritis in humans and other mammals [11]. These findings are in agreement with the works of Turan *et al*. (2007) [12]; Valentine *et al*. (2018) [13] and Rajesh and Vikas, (2014) [14].

The extracts were tested on several strains of fungi. The ethanolic extract showed the highest zone of inhibition in *Aspergillus flavus* (15mm), *Microsporum ferrogenium* (12mm) and *Mucor mucaralis* (9mm) but lowest in *Tricophyton tonsurance* (4mm). For methanolic extracts, the zone of inhibition was highest in *Microsporum ferrogenium* (13mm) and *Mucor mucaralis* (11mm) but lowest in *Tricophyton tonsurance* (5mm). For ethyl acetate extract, the zone of inhibition was highest in *Aspergillus flavus* (16mm), *Mucor mucaralis* (14mm) and *Microsporum ferrogenium* (12mm) as shown in table 3 above. These findings are in concord with Senanayake *et al*. (2010) [15] and Sunita *et al*. (2017) [9]. The inhibition of bacterial and fungal growth at different degrees by the plant extract is an indicator that the plant extract is an effective antimicrobial and antifungal agent and can be used in alternative medicine in the prevention, treatment, management and control of a variety of both drug resistant and non-resistant bacterial and fungal species.

Conclusion

From the present study, it can be inferred that the methanol, ethanol and ethyl acetate extracts of *Brassica juncea seeds is* an effective antimicrobial and antifungal agent against pathogenic microbes. The result of the study supports the immense medicinal properties of *Brassica juncea seed* which has revealed a significant scope to develop a novel broad spectrum of antimicrobial herbal formulation. This study paves the way for further attention and research to identify the active compounds responsible for the plant's biological activity. Further studies to elucidate these bioactive components and their exact mechanism of action are highly recommended.

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