Antibacterial Activity Of Different Plant And Callus Extracts A Comparative Study

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Abstract: Current study includes antibacterial activity of different plant and callus extract such as Momordica charantia (Karela), Cucurbita pepo (Pumpkin), Capsicum annum (Chilli), Coriander sativum (Coriander), Brassica nigra (Mustard), Nigella sativa (Black Cumin), Trigonella foenum (Fenugreek) against eight pathogenic bacteria of which five are gram positive such as Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Streptococcus sp, Bacillus megaterium and three are gram negative such as Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa. Plant materials were extracted using ethanol. All the extracts showed zone of inhibition against these pathogenic bacteria which were tested by agar well diffusion method. The leaf extract of Momordica charantia, Nigella sativa, Barassica nigra showed good zone of inhibition as compared to other extracts against S.aureus (13mm, 17mm,11mm), E.coli (13mm,10mm,18mm), P. aeruginosa (11mm,12mm,10mm), Streptococcus (14mm, 15mm, 10mm), B.subtilis (10mm,17mm,10mm), P. vulgaris (15mm,18mm, 10mm), B.cereus (13mm,16mm,12mm), B.megaterium (12mm,15mm,10mm). Explants from in vitro grown plants were cultured on MS-Medium with different combination of IAA and Kinetin for callus induction. Among those combination of IAA and Kinetin (0.1 x 0.0), (1.0 x 0.0), (0.4 x 0.5), (1.0 x 0.5), (1.5 x 0.5), (0.4 x 1.0), (0.8 x1.0), (0.1 x 1.5) mg/L have showed maximum growth of calli.

Keywords: Pathogens, plant extract, MS-Medium, plant growth regulators, antibacterial activity.

Introduction

Nature has wide sources of medicinal agents and many drugs are isolated from nature. Plant based medicinal system play an important role in health care. Medicinal plants would be the best source to obtain variety of drugs. Traditionally man uses variety of drugs to cure diseases. For example, use of turmeric (Curcuma longa) to treat flu, cough and nasal infection. With the industrial development, chemical products are widely used to treat diseases. But these chemical products have some side effects. So people become aware about these side effects and are now turned to traditional biological sources to cure diseases. People try to reduce the use of chemical products/drugs and searching for natural products, which have no side effects compared to chemical drugs.

Materials and methods

Sample collection: Plant leaves and seeds of Capsicum annum, Momordica charantia, Coriander sativum, Trigonella foenum, Cucurbita pepo and seeds of Nigella sativa, Brassica nigra were collected from local farm and market. Leaves after proper washing were dried in hot air oven for 2-3 days then ground into powder and seeds were ground into powder properly.

Extraction: Fifty grams (50g) each of the powdered plant material was soaked in 100 ml of ethanol in separate round bottom flask. Extraction was carried out by using Reflux Method. The mixture obtained was filtered by using sterile cheese cloth. Filtrate was evaporated at room temperature.

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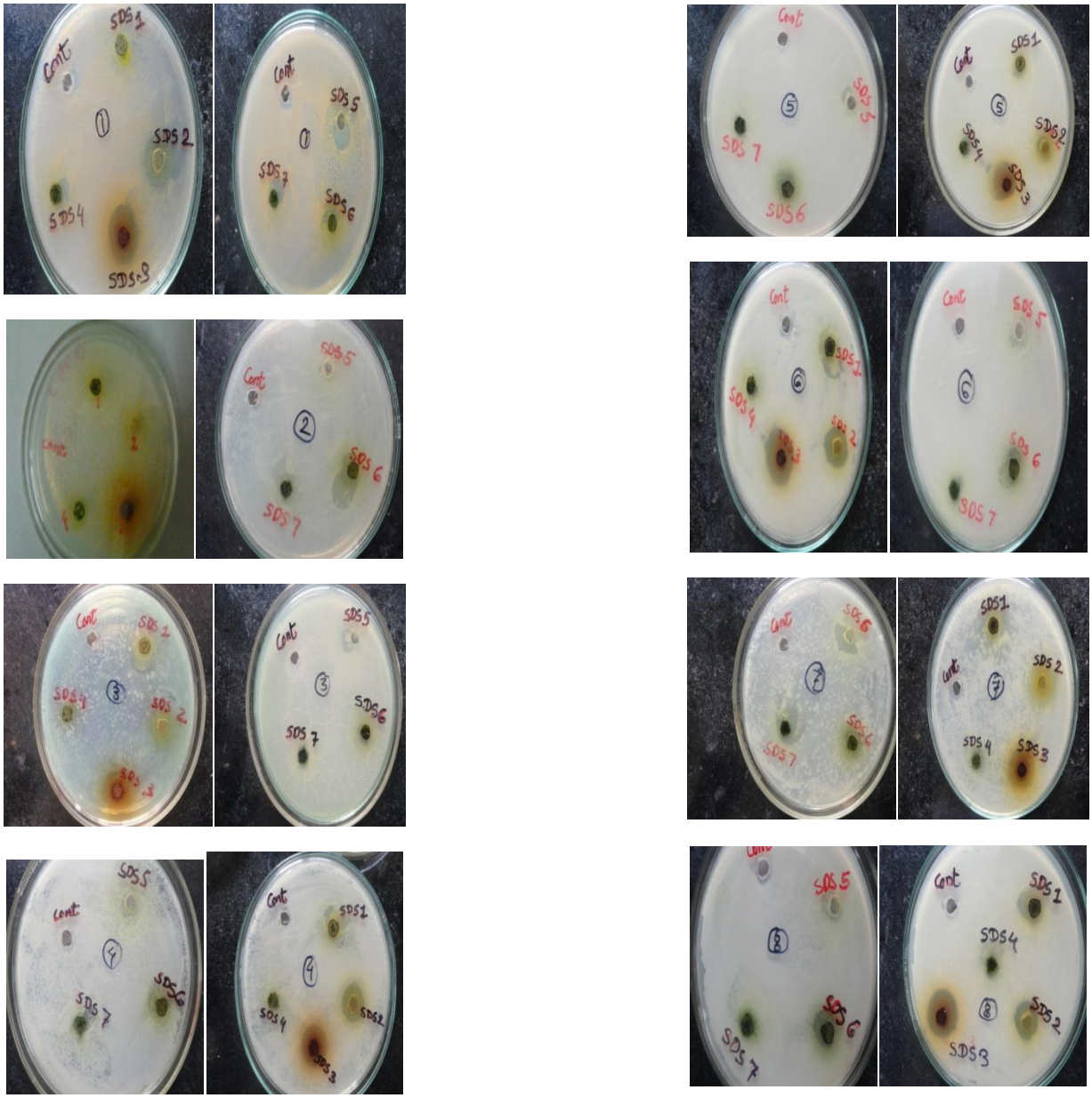
Antibacterial assay: Antibacterial assay was done by agar well diffusion method. Suspension of eight pathogenic bacteria such as Staphylococcus aureus, Escherichia coli, Bacillus cereus, Streptococcos sp, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa, Bacillus megaterium swabbed on to Nutrient Agar in separate petri dishes. Wells were prepared on the Agar plate with the help of borer. 50 µl of plant extract was poured in the wells. Ethanol was used as control. The plates were incubated at 37^{o}C for 24 h. After incubation, zone of inhibition (mm) was measured to determine antibacterial activity of the different extracts used[1][2][3].

Seed germination: Collected seeds were germinated by different methods such as MS medium, wet filter paper, moist soil, breaking seed dormancy.

Callus induction: MS-Media was prepared with addition of hormones and sterilized. In vitro grown plant parts of Brassica nigra were cut into explants, were then inoculated into MS-Medium. Incubation was carried out at 25^{o}C. Different concentrations of IAA and kinetin were used for callus induction[4].

Antibacterial activity: All the extracts were not active against pathogenic bacteria tested. The leaf extract of Capsicum annum was active against tested pathogenic bacteria but differ in zone of inhibition and showed maximum zone of inhibition against P.vulgaris i.e. 13mm. The leaf extract of Momordica charantia was mostly active against Streptococcus sp. and P.vulgaris i.e. 14 and 15mm. The seed extract of Nigella sativa was found to be most active against all pathogenic bacteria. The leaf extract of Coriander sativum was active only against S.aureus and E.coli. The seed extract of Brassica nigra was active against tested pathogenic bacteria and showed maximum zone of inhibition against B.cereus i.e. 12mm. The leaf extract of Trigonella foenum was not active against P.aeruginosa and most active against E.coli. The leaf extract of Cucurbita pepo was not active against P.aeruginosa, B.subtilis and P.vulgaris.

Fig: 1 Antibacterial activity of several plant extracts on various pathogens

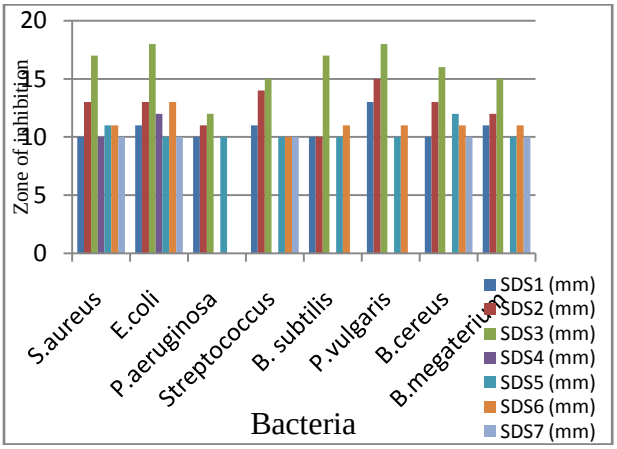


SDS1**:** Leaf extract of Capsicum annum, SDS2: Leaf extract of Momordica charantia, SDS3: Seed extract of Nigella sativa, SDS4: Leaf extract of Coriander sativum, SDS5: Seed extract of Brassica nigra, SDS6: Leaf extract of Trigonella foenum, SDS7: Leaf extract of Cucurbita pepo

Table 1 Antibacterial assay of plant extracts on different human pathogens kinetin

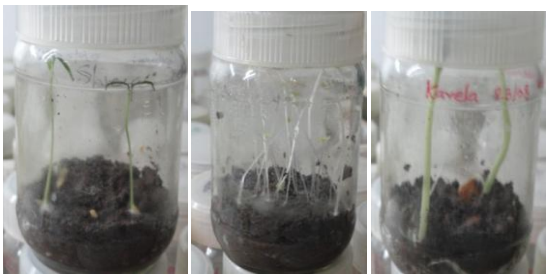
Bacteria	SDS1 (mm)	SDS2 (mm)	SDS3 (mm)	SDS4 (mm)	SDS5 (mm)	SDS6 (mm)	SDS7 (mm)
S.aureus	10	13	17	10	11	11	10
E.coli	11	13	18	12	10	13	10
P.aeruginosa	10	11	12	0	10	0	0
Streptococcus sp	11	14	15	0	10	10	10
B.subtilis	10	10	17	0	10	11	0
P.vulgaris	13	15	18	0	10	11	0
B.cereus	10	13	16	0	12	11	10
B.megaterium	11	12	15	0	10	11	10

Fig.2 Zone of inhibition of plant extracts against pathogenic bacteria



Seed germination: The seeds of Cucurbita pepo, Momordica charantia have hard seed coat so these seeds were germinated by breaking their seed dormancy by soaking them in 50% conc.H_2SO_4 for 1 h. Seeds of Capsicum annum were germinated on MS- medium, moist soil, wet filter paper. Seeds of Brassica nigra were germinated on moist soil and wet filter paper.

Fig.3 Aseptic seed germination in moist sterile soil



Capsicum annum Brassica nigra Momordica charantia

Fig. 4 Callus induction on MS media with various concentrations of IAA and kinetin

Initial callus formation was observed within two weeks. The callus was soft, brownish in colour. Different concentrations of IAA and Kinetin were used, among them (0.1 x 0.0), (1.0 x 0.0), (0.4 x 0.5), (1.0 x 0.5), (1.5 x 0.5), (0.4 x 1.0), (0.8 x 1.0), (0.1 x 1.5) mg/L have showed maximum growth of calli. So these concentrations were used for further callus induction. Contamination was observed in 1 or 2 culture bottles.

Table 2 Callus induction by using different concentrations of IAA and kinetin

IAA mg/L x Kinetin mg/L	0.1	0.4	0.8	1.0	1.5
0.0	0.1 x 0.0 (+)	0.4 x 0.0 (c)	0.8 x 0.0 (c)	1.0 x 0.0 (+)	1.5 x 0.0 (+)
0.5	0.1 x 0.5 (+)	0.4 x 0.5 (+)	0.8 x 0.5 (+)	1.0 x 0.5 (c)	1.5 x 0.5 (+)
1.0	0.1 x 1.0 (c)	0.4 x 1.0 (+)	0.8 x 1.0 (+)	1.0 x 1.0 (+)	1.5 x 1.0 (c)
1.5	0.1 x 1.5 (+)	0.4 x 1.5 (-)	0.8 x 1.5 (-)	1.0 x 1.5 (c)	1.5 x 1.5 (+)
2.0	0.1 x 2.0 (+)	0.4 x 2.0 (c)	0.8 x 2.0 (c)	1.0 x 2.0 (c)	1.5 x 2.0 (c)

(+) indicates growth, (-) indicates no growth, (c) indicates contamination

Discussion:

Seeds of Momordica charantia, Cucurbita pepo have hard seed coat, so to germinate them fast seed dormancy was broken by 50% conc. H_2SO_4. All the composition used for callus induction did not show the growth of callus, so composition (0.1 x 0.0), (1.0 x 0.0), (0.4 x 0.5), (1.0 x 0.5), (1.5 x 0.5), (0.4 x 1.0), (0.8 x 1.0), (0.1 x 1.5) mg/L of IAA and kinetin were selected which showed the healthy growth of callus. The plant extract of Brassica nigra and Momordica charantia have showed good zone of inhibition as compare to other plant extract, so they may have good medicinal properties.

References

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