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Original Research Article

Study of *Daucuscarota ssp. Sativus* and *Butea monospermato* analyse their Applicability in Pharmaceutical Industry as Antimicrobial Agents

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Abstract

Human Beings have been using plant products to heal the Wounds and Diseases from the inception of humankind. Even when it was not known that microorganisms exist, People have been using antimicrobial agents prepared from plants. These antimicrobial products were prepared by extracting the plant in a suitable solvent. Antimicrobial property is conferred to plants by the presence of various phytochemicals which are the products of several Secondary metabolic pathways. The aim of this project was to decipher the potential use of *Daucuscarota ssp*. *Sativus* and *Buteamonosperma* in pharmaceutical industry. In this research, Qualitative phytochemical screening and antimicrobial potential of Black carrot and Kamarkas has been studied. Black carrot showed good antimicrobial activity against *A. brasiliensis, E. coli* and *S. enterica*, arranged in descending order of the Slope obtained in each antimicrobial assay. Phytochemical screening showed the presence of Flavonoids, Soluble Phenolic Compounds, Naphthoquinone and traces of Saponins and Alkaloids. The Kamarkas showed antimicrobial activity against S. aureus and to some extent against *A. brasiliensis*. Phytochemical analysis of Kamarkas showed positive for all phytochemicals.

Keywords: Phytochemical screening; Antimicrobial activity; Black Carrot; Kamarkas

Introduction

From more than a thousand years people have been using plants, in one way or the other to cure diseases. Within Asia, there are a number of system of medicines which are still evolving. For example, Ayurveda, which is indigenous to India, traditional Chinese medicine, Unani from Islamic countries and many are still undiscovered. Today, when so many countries are economically weaker and their people are dying of diseases, it looks like a very good idea and very convincing one that a disease can be cured by folk medicines. For many years, modern scientific community has underestimated the treasure that has been used and refined from so many years by millions by traditional Ayurvedacharyas and ancient Medicinal Practitioners. For someone who cannot afford treatment of a debilitating disease at a modern hospital, ancient medicine is a ray of sunlight in a dark and suffocating room. In this project, the focus is to assess the antimicrobial activity of certain medicinal plants for some selected very disease Causing strains of bacteria. Testing for such strains will provide us to find novel methods for treating such diseases. The active compounds in plant that confer antimicrobial activity are Phytochemicals or Secondary Metabolites. These compounds, which are mostly aromatic or oxygen substituted derivatives give plants their characteristic antimicrobial activity. These substances form a system that is analogous to immune system in plants as these substances help in prevention of predation by pathogenic organisms [1] [2] [3] . In this project the emphasis is given to assess the antimicrobial activity of Ethanolic plant extracts through well diffusion.

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Daucuscarota ssp. Sativus (Black carrot)

This plant has edible root and it has been cultivated from thousands of years. Daucuscarota wildtype originated in Afghanistan and then spread to Europe and rest of the Asia and then to America [4] . In India, Red variety of carrot is mostly used, Black carrot in India are consumed mostly in winters till Holi, which is a festival of Colors celebrated in the month of march. It is a rich source of anthocyanins that is responsible for its characteristic Purple-dark Red colour. Black Carrothave high antioxidant activity due to presence of anthocyanins in good amounts [5] . Anthocyanins are a type of flavonoids which possesses Antioxidant and antimicrobial activity. Anthocyanins are good for neural disorders and it can also neutralise the toxins which can impair the normal cells during chemotherapeutic treatment of cancer [6] [7] . Black Carrot can be considered as a good candidate for producing red/pink/purple food colours as a better and acceptable alternative instead of carmine from Arthropods.

Butea monosperma (Kamarkas)

Butea monosperma is also known as Flame of Forest, Palash, Dhak, Bengal Kino, Bastard Teak, Chichra, Kamarkas etcetera. Kamarkas can be used to heal the abdominal muscles, stretch marks, and to increase milk production after delivery, according to Ayurveda. Kamarkas is widely distributed in India, Nepal, Srilanka, Burma, lower Himalayas, KhandeshAkrani region of South India. The flowers of this tree appear in February and persist till April end [8] .This tree is a very slow growing deciduous tree. The height of this tree on an average reaches 12 to 15 meters. It has irregular branches and warped trunk. Flowers of this tree are orange-red in colour due to presence of pigment compounds such as Chakones and Aurones. Various compounds other than primary metabolites have been isolated and characterized from its flowers and other parts of the tree. There is a high possibility for such compounds to be present in the butea gum also. Butea gum is granular, dark reddish brown in colour with bits of bark. The taste of the gum is very strong and leaves a sensation of dryness in mouth indicating that there are some volatile compounds present in it. Bark is ash in colour. Different parts of this plant have been used in folklore medicines from hundreds of years for conditions such as: Impotency, Infertility, Dysentery etc. Apart from being used as a medicine or a condiment, the wood of this tree has been in use for the manufacturing of furniture but, the wood is vulnerable to get infested by lac insect. Flower, seeds, bark have already been investigated and it is well known that all of them have antimicrobial activity [8].

Material and Methods

Plant samples

Root of Daucuscarota Ssp. Sativus (Black carrot), Gum of Butea monosperma (Kamarkas)

Chemicals

Ethanol (99.9%), Distilled Water, Concentrated sulfuric acid, Chloroform, Concentrated Hydrochloric acid, Dimethyl sulfoxide, 10% Sodium hydroxide, 10% Ferric chloride, 5% Ferric chloride, 10% Potassium hydroxide, Wagner's reagent, Acetonitrile

Media

Nutrient agar powder

Chloramphenicol yeast glucose agar

Maximum recovery diluent

Streptomycin S^{10} disks

Microorganisms

All Microbes Used were obtained from FICCI Research and analysis centre:

Aspergillus brasiliensis

Escherichia coli

Salmonella enterica

Staphylococcus aureus

Qualitative Phytochemical screening

This was done to qualitatively assess the presence of various secondary metabolites in the aqueous extract of plants. These tests were adopted from a paper by Vinoth B. et al on Antibacterial properties of Azadirachta [3] . 2g of dried and ground sample was mixed with 30 ml DW in a bottle with lid and incubated in water bath for 30 minutes at 100°C. The bottles were then cooled down and transferred to shaker for about 24 hours. After 24 hours, the liquid was separated using filter paper. 20 ml water was also added to make them pass through filter paper easily because the extracts were viscous.

Saponins: Water and Aqueous extract were mixed in equal proportion and vigorously shaken. Appearance of foam, confirms presence of Saponins.

Flavonoids: Extract and 10% Sodium hydroxide was taken in a ratio of 1:2, on addition of Hydrochloric acid Yellow colour formed in first step disappears.

Tannins: Extract was treated with few drops of $FeCl_3$, appearance of blue / black / brown / green precipitate confirms the presence of Tannins.

Soluble Phenolic Compounds: A small amount of extract was treated with 5% $FeCl_3$, appearance of deep blue colour confirms the presence of SPCs.

Terpenoids: A small amount of extract was mixed with double volume of chloroform, followed by treatment with Concentrated Hydrochloric acid appearance of red-brown/violet colour confirms the presence of Terpenoids.

Naphthoquinone: Extract and 10 % Hydroxide was mixed in a ratio of 3:1, appearance of blue-Black colour, confirms the presence of Naphthoquinone.

Alkaloids: Extract was treated with Wagner's reagent, appearance of red / orange / brown precipitate confirms the presence of alkaloids.

Extract preparation and Well diffusion Antimicrobial assay

Black Carrotwas first washed with tap water, cut into thin pieces, then placed on aluminium foil in a single layer and dehydrated in an incubator at 60°C in dark conditions. Kamarkas was obtained as a dried granulated powder. The dehydrated samples were then ground with the help of a kitchen blender to a fine powder. A maximum quantity of this powder was then transferred to the self-made thimbles of filter paper and then weighed. These thimbles were then used for Soxhlet extraction at 80°C with ethanol as solvent for 24 hours. After 24 hours, the apparatus was turned off and the solvent was evaporated by keeping the flask on boiling water bath until amorphous solid extract was obtained. This concentrate was then weighed and accordingly, a known quantity of DMSO was added until it got partially suspended and then diluted with water to make a solution with maximum extract concentration and with 4 % DMSO in it. This Extract was then autoclaved with usual procedure.Autoclaving is a better method of sterilization of extracts than aerodisc syringe filtration [11] . From this extract, three more extract solutions were prepared with different concentrations in 4% DMSO in sterile water. Petri plates with Nutrient agar and Chloramphenicol yeast glucose agar were prepared for antibacterial and antifungal assays. The plates were spread with 100 μl of inoculum, prepared by pouring 9 ml of Maximum Recovery Diluent in cell revival slants and vortexed for 40 seconds.Then, wells were made with the back of micro tip, and each well was labelled with different concentrations. To the labelled wells, extracts with varying concentrations were added, Positive control with Streptomycin S^{10} disks and negative control with 4% DMSO were also set. Bacterial plates were incubated at 37°C for 24 hours and fungal plates were incubated at 25°C. After visible growth, the zones of inhibition were measured around each well with Vernier callipers and data was recorded for each concentration. Finally, Concentration Versus IZD area graph was plotted for each sample. Slopes were determined for the "line of best fit" to the data. Slopes in the given data shows the extent of effect of changing concentrations of the plant extract on the IZDs observed.

Results & Discussion

From Soxhlet extraction at 80°C, for 24 hours the mass percentage that can be extracted from dry mass of plant sample was determined using following formula:

$$\text{Percentage of Extraction} = \frac{\Sigma W' t - \Sigma W}{S} \times 100$$

Where, W' is the Weight of flask after extraction and solvent evaporation, W is Weight of empty flask, S is the Weight of sample in thimble. It was found that 43.4 % of dry black carrot mass can be extracted using ethanol. Whereas 34.2% of dry Kamarkas mass can be extracted using ethanol as the solvent ofextraction. Ethanol was selected as the solvent for extraction as it has the capability to extract saturated organic compounds, aromatic compounds viz. polyphenols in good amounts; Second, Ethanol also degrades the enzyme polyphenol oxidase which is active in water and oxidises polyphenols;thirdly, it can easily penetrate the plasma membrane to extract intracellular ingredients from plant material [9] . After extraction and evaporation of ethanol, the concentrate was re-dissolved in 4% DMSO. DMSO was selected because it has less toxic effects against microorganisms and it can dissolve polar as well as non-polar compounds due its inherent polarity and aprotic nature [10] . Kamarkas showed the presence of all phytochemicals tested: Saponinns, Flavonoids, Tannins, Soluble Phenolic Compounds, Terpenoids, Napthoquinone and Alkaloids whereas, Black carrot showed the presence of Flavonoids, Soluble phenolic Compounds, Napthoquinone and traces of Saponins and Alkaloids (Table 1).

Table 1 Result of Qualitative phytochemical screening.

	Control	Black Carrot	Kamarkas
Saponins	-	Trace	+
Flavonoids	-	+	+
Tannins	-	-	+
Soluble Phenolic compounds	-	+	+
Terpenoids	-	-	+
Napthoquinone	-	+	+
Alkaloids	-	Trace	+

Alcoholic extract of Black Carrot showed good antimicrobial potential against E. coli, S. enterica and A. brasiliensis. The degree of effect of Concentration on IZDs in microbial lawns is highly pronounced in the case of A. brasiliensis, followed by

E. coli and least in S. enterica, as indicated by the slopes of the line of best fit. Antibacterial activity (IZDs) was found to be directly dependent on the concentration of black carrot extracts.Phytochemical analysis of Black carrot showed positive for: Flavonoids, Soluble PhenolicCompounds, Naphthoquinone and traces of Saponins and Alkaloids. The values of IZD at different concentrations, Slopes in the respective bacteria along with positive and negative controls are as shown in the Table 2 and Figure 1.

Table 2 The IZDs observed atdifferent concentration of Ethanolic Black Carrot extract, Positive control andNegative control observed in the microbial lawns of E. coli, S. enterica and A.brasiliensis.

Concentration	E. coli. IZD (mm)	S. enterica IZD (mm)	S. aureus IZD (mm)	A. brasiliensis IZD (mm)
75%	27.8	26.6	9	26.2
50%	24.1	22.4	-	24
25%	20.6	20.4	-	14
10%	16.5	18.4	-	10.2
Slope	16.73	12.12	-	26.73
Streptomycin S10 (+ Control)	25.4	24.05	24.9	0
4% DMSO (- Control)	0	0	0	0

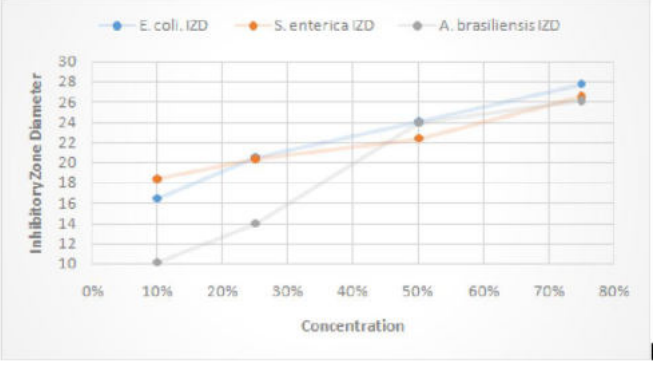

Figure 1 This graph shows theeffect of variable concentration of black carrot Ethanolic extract on theInhibitory zone diameters on Microbial lawns of E.coli, S. enterica and A.brasiliensis.

Alcoholic extract of Kamarkas showed significant antimicrobial activity against S. aureus and Slightly against A. brasiliensis. The slope in case of S. aureus is not very steep as evident by Figure 2 therefore the extent of effect of increase in concentration on IZDs on microbial lawns is not very high. Phytochemical analysis of Kamarkas showed positive in all tests performed [11] .

Table 3 he IZDs observed atdifferent concentration KamarkasEthanolicExtract, Negative control observed inthe microbial lawns of S. aureus and A. brasiliensis and the slope observed inthe Data.

Concentration	E. coli. IZD (mm)	S. enterica IZD (mm)	S. aureus IZD (mm)	A. brasiliensis IZD (mm)
30.20%	-	-	22.7	12.3
20%	-	-	21.5	0
10%	-	-	21.3	0
5%	-	-	19.6	0
Slope	-	-	10.53	-
Streptomycin S10 (+ Control)	25.4	24.05	24.9	0
4% DMSO (- Control)	0	0	0	0

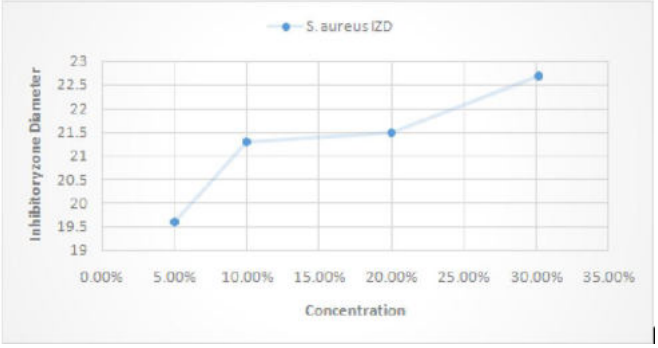

Figure 2 This graph shows theeffect of variable concentration of Kamarkas alcoholic extract on theInhibitory zone diameters on Microbial lawns of S. aureus

Conclusions

Antimicrobial potential of Ethanolic Extract of Black Carrot was observed to be higher than Kamarkas. Black Carrot showed good antimicrobial potential against E. coli, S. enterica and A. brasiliensisas shown by slope in table 2 and figure 1, whereas Kamarkas showed antimicrobial potential only against S. aureusas shown by slope in table 3 and figure 2.Therefore, use of Black Carrot in pharmaceutical industry as potential antimicrobial agent should be considered. This antimicrobial effect of Black Carrot was probably due to presence of soluble phenolic compounds as indicated by Qualitative phytochemical screening, most likely anthocyanins in huge amounts.

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