



Anti-bacterial studies and identification of anti-oxidant fraction in two varieties of pepper

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Abstract

The present in-vitro pharmacological studies aims for the detection of the biological activity of methanolic and chloroform extracts of the two species of pepper. Anti-microbial assay was carried out in the chloroform extract of black pepper and long pepper and the *in vitro* DPPH assay was carried out for both the methanolic and chloroform extract of both the species. In case of the microbial activity the chloroform extract was potential and in the in vitro antioxidant studies, the methanolic extract of black pepper (EC₅₀ 60,130,180) only possess the activity The antibacterial study was carried out using two species of pepper in chloroform extract which shows more activity against *S typhi* compared to other organism.

Keywords: Pepper, DPPH, Antioxidant, Antibacterial activity.

Introduction

Herbs and Spices have been used for thousands of years to enhance the flavor, color and aroma of food; additionally, they are known for their preservative and medicinal value (Beuchat *et al*, 1994; Middha *et al*, 2009; Cutler,1995) . Black pepper (*Piper nigrum* L., Piperaceae) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion and fever (Beuchat and Golden,1989). It has been shown that Piper has antimicrobial activity(Bawa,1992) and some have already produced compounds, effective against antibiotic resistant strains of bacteria(Hamrick *etal*,1992). *Piper longum* L. is popularly known as “pippali” or “long pepper”. It is an important medicinal plant in Indian traditional medicine. It is distributed throughout the Indian subcontinent (Kirtikar and Basu, 1933). Flowers are yellow, in elongate spikes and the fruits are small, ovoid berries, shiny blackish green, embedded in fleshy spikes (Williamsons, 2002). They have a pungent taste and cause salivation and numbness of the mouth(Neelam and Krishnaswamy Kamala,2001) .

Long pepper is very effective in the treatment of bronchial asthma in children(Dahanukar *et al*,1984; Anshuman *et al*,1984) It has been reported that the fruits extract of the plant had antidepressant, antinociceptive, anti- inflammatory, antioxidant, anticancer, antidiabetic, antibacterial, antifungal, antitumor, anti-allergic, antiasthmatic, antifertility, antiulcer, antihypertensive, antiplatelet, anti-thyroid, immunomodulatory, antiamoebic, hepato- protective, vasodilating, insecticidal and mosquito larvicidal activities(Zaveri *et al*,2010;Manoj *et al*,2004; Singh *et al*,2009) . Roots and fruits are also used as anti-dote in snake biting and scorpion sting (Chopra *et al* ,1956). *P. longum* L. has been used in traditional remedies as well as in the Ayurvedic system of medicine against various disorders (Tripathi *et al*, 1999)

Materials and Methods

DPPH Assay

The capacity of the extracts to scavenge the stable 2,2'-diphenyl-2-picrylhydrazyl (DPPH) free radical

was measured (Duan *et al.*, 2007). Antiradical activity was measured by a decrease in absorbance at 517 nm of a solution of colored DPPH in methanol brought about by the sample. A stock solution of DPPH (1.3 mg/ml) was prepared such that 75µl of it in 3 ml methanol gave an initial absorbance of 0.9. The decrease in the absorbance in the presence of sample extract and standard at different concentrations were noted after 30 minutes. EC₅₀ was calculated from % inhibition. A blank reading was taken using methanol instead of sample extract. Absorbance at 517nm is determined after 30 minutes using UV –visible spectrometer and IC₅₀ (Inhibitory concentration to scavenge 50% free radicals) is also determined. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. IC 50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The capacity to scavenge to DPPH radical was calculated using the following equation (17).

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

where,

C = absorbance of DPPH alone

T = Absorbance of DPPH along with different concentrations of extracts

IC₅₀ was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition.

Antibacterial study

The given liquid was made up to different concentration as considering the liquid as stock. Overnight bacterial suspension (100µl) adjusted to contain 1x10⁶ CFU/ml of bacteria, spread by a sterile glass rod on Nutrient Agar (NA) medium. The inoculated plates were incubated at 27±20C for 24 h, and then the inhibition zones were measured in diameter (mm). Antibiotic discs containing 1 µg of Ciproflaxin CF1 was used as positive controls and

DMSO used as negative control. The MIC was calculated using the 4 concentrations of sample.

Results and Discussion

The DPPH scavenging activity of chloroform and methanolic extract of both the species were carried out. The black pepper in methanolic extract showed three EC 50 values which is shown in graph1. The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity (Brand Williams *et al* 1995; Katalinic *et al*, 2004). DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al.*, 2002). It is accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen donating ability. The collected fruit extracts exhibited remarkable DPPH free radicals scavenging ability at different concentrations. From these, the % inhibition concentrations and IC₅₀'s were calculated. From the previous studies it is reported that the antioxidant and radical scavenging activities of black pepper (*Piper nigrum* Linn.) seeds have been well reported (Gulcin, 2005). Both water extract and ethanol extract of black pepper exhibited strong antioxidant activity.

The antibacterial study was carried out using black pepper and long pepper in chloroform extract which shows more activity against *S typhi* compared to other organism used shown in table 1 and 2. From the previous study the other organisms used are *Escherichia coli*, *Staphylococcus aureus* and *K. pneumoniae*. Chloroform extract of *Piper nigrum* showed high antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Proteus* sp. and *Staphylococcus aureus* where as *Pseudomonas aeruginosa* showed resistant. The chloroform extract of *Piper nigrum*, showed maximum inhibition against *Escherichia coli* and *Staphylococcus aureus* and *Salmonella typhi*. *Pseudomonas aeruginosa* was resistant to chloroform extract of *Piper nigrum* (23).

Graph: 1 DPPH Assay

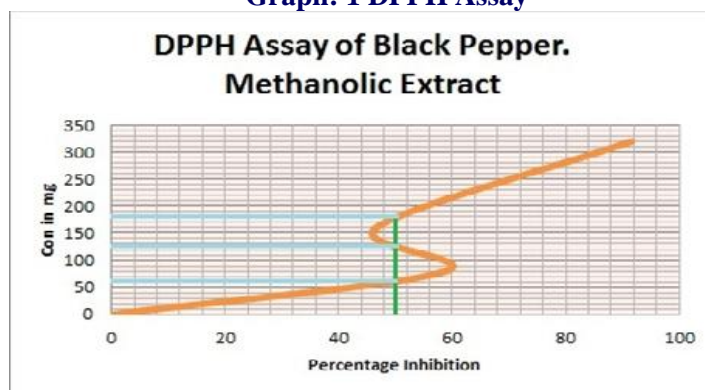


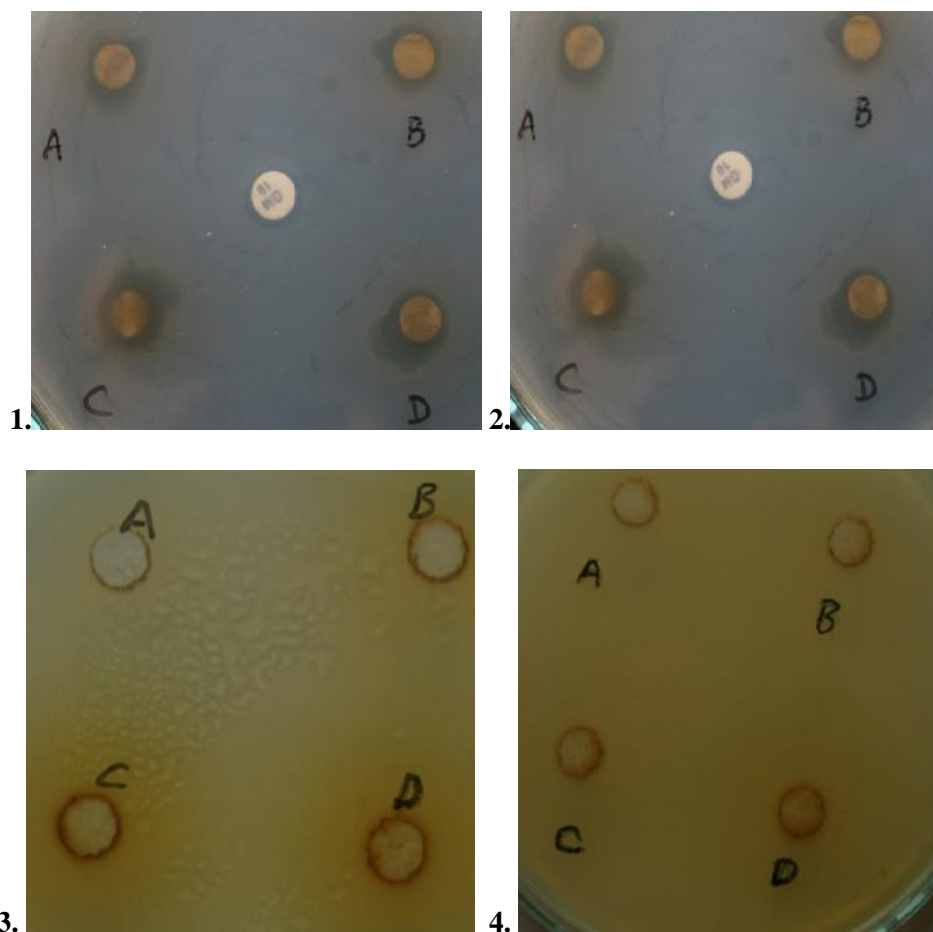
Table: 1 Black pepper in chloroform extract

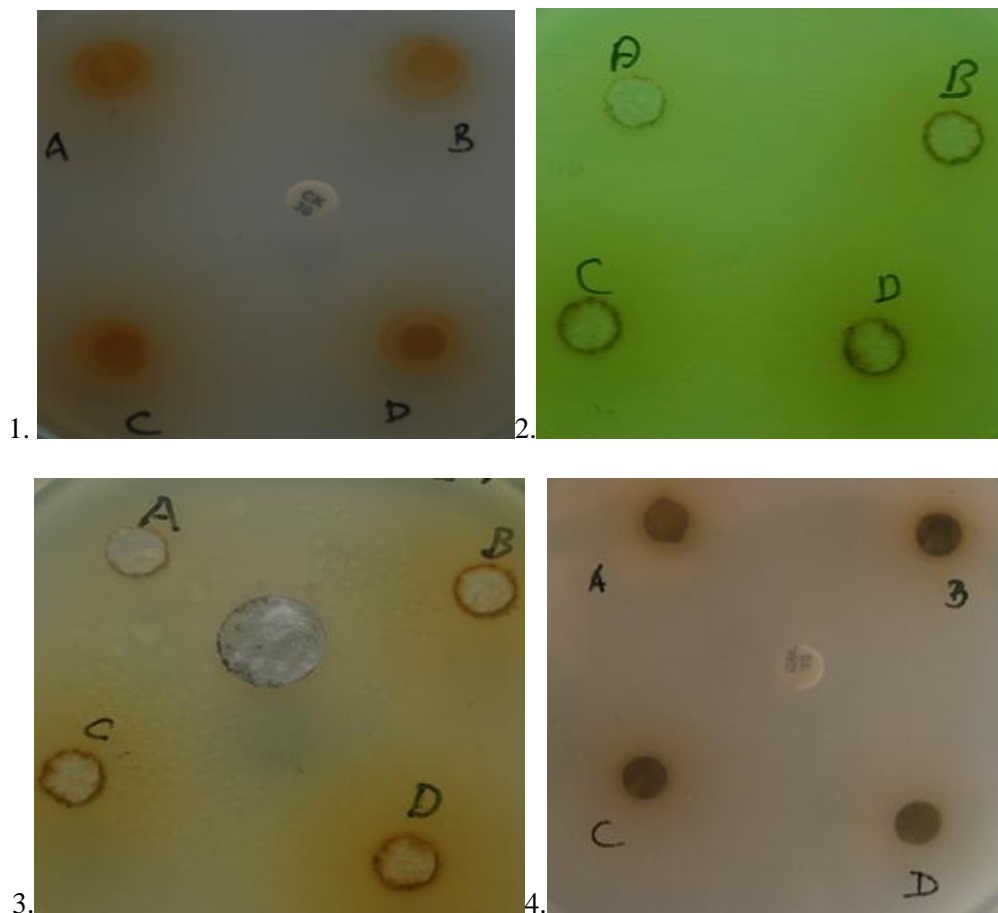
Test organisms	Concentration of the sample					Ciprofloxacin 5 µg
	25	50	100	150	MIC	
<i>Escherichia coli</i>	0 mm	0 mm	0mm	2mm	150	15mm
<i>Staphylococcus aureus</i>	0 mm	0 mm	2mm	4mm	100	20mm
<i>S. typhi</i>	0mm	2mm	2 mm	4mm	50	25mm
<i>K. pneumoniae</i>	0mm	0mm	2mm	2mm	100	20mm

Table:2 Long pepper in chloroform extract

Test organisms	Concentration of the sample					Ciprofloxacin 5 µg
	25	50	100	150	MIC	
<i>Escherichia coli</i>	0 mm	2mm	4mm	4mm	50	15mm
<i>Staphylococcus aureus</i>	0 mm	2 mm	2mm	4mm	50	20mm
<i>S. typhi</i>	2mm	2mm	2 mm	4mm	25	25mm
<i>K. pneumoniae</i>	0mm	0mm	2mm	2mm	100	20mm

Diagram: 1 Black pepper in chloroform extract





Conclusion

From the results it was observed that the chloroform extract was carried out for antibacterial assay as it was rich in biochemical parameters and also the chloroform extract of the black pepper and long pepper was more active against the *S. typhi* when compared to that of the other organisms. The superoxide scavenging assay was carried out in both the methanol and chloroform extract of two species and found that the methanolic extract of the black pepper only shows significant EC 50 values at 3 doses of 60,130,180. Thus we finally conclude that the black pepper in alcoholic possess significant activity so this may be reason for the usage of the same in kashayas which are commonly used in Ayurvedic system of Medicine.

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