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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON CAPSICUM EXTRACTS

VASAVI S[1*], GAYATHRI N[1*], JOYSE RL[1*], VASANTHA S[1*], PANDY V[2] AND NADENDLA R[3]

1: UG Scholar, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Andhra Pradesh 522034

2: Professor, Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Andhra Pradesh 522034

3: Principal, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Andhra Pradesh 522034

***Corresponding Author: Dr. S. Vasavi: E Mail: singamanenivasavi@gmail.com, Tel: 9063528925**

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ABSTRACT

Background: The compounds which are present in plants and obtained by primary or secondary metabolism are known as phytochemicals. These phytochemicals act either as a drug or as a source for preparation of drugs Capsicum contains many useful phytochemicals such as flavonoids, carotenoids and phenolics and claimed to be responsible for antioxidant activity. This property of capsicum can protect the body from free radicals and oxidation process. The present study was aimed to perform a comparative phytochemical and pharmacological study with focus on analgesic activity using mouse models.

Materials and Methods: Three different capsicum varieties such as green chilli, red chilli and capsicum (Capsicum annum belongs to the family Solanaceae) were grinded into coarse particles using mechanical grinder and subjected to methanolic extraction.

Results and Discussion: Percentage yield of methanolic extract of 3 different varieties such as green chilli, red chilli and capsicum was found to be 8.9, 17.8, and 10% w/w respectively. These methanolic extracts were further subjected to phytochemical screening.

Conclusion: This study results revealed that all three varieties of capsicum (green chili, red chili and capsicum) possess similar phytochemicals such as carbohydrates, alkaloids and tannins. These phytochemicals could be responsible for various pharmacological activities including analgesic activity of capsicum varieties.

Keywords: Phytochemicals, Flavanoids, Free Radicals, Carotenoids, Phenolics, Solanaceae, Phytochemical screening, Mechanical Grinder

INTRODUCTION

Pain: It is "an unpleasant sensory and emotional experience, associated with actual or potential tissue damage. Pain is a physical and emotional experience **[1]**. Based on duration pain is divided into 2 types as acute and chronic pain. Acute pain is directly related to soft tissue damage. It lasts less than 3 to 6 months **[1]** (abstract). Chronic pain persists for a long period of time **[2, 3]**. Based on the source pain, it is categorized into two types as nociceptive and neuropathic pain.

Treatment of Pain: Pain can be treated in 2 ways either by opioid treatment or nonopioid treatment. Opioid treatment means use of opioid analgesics such as morphine, and tramadol and non-opioid analgesics such as NSAIDS, corticosteroids and anti-depressants etc. The pain can be also managed with physical therapy, acupuncture, and surgery.

Capsicum: Phytochemical is the word obtained from the Greek word 'Phyton' which means plant. Phytochemicals are the chemical compounds obtained from plants by primary or secondary metabolism. Capsicum is also known as bell pepper, chilli, Chile pepper, sweet pepper etc., belongs to family Solanaceae. It contains more than 30 species of flowering plant **[4]**. Out of them 5 different varieties are widely cultivated which includes *C. annuum* L., *C. chinensis* Jacq., *C. frutescens* L., *C. pubescens.*, and *C. baccatum* L. Peppers are mainly used as flavourants and colourants. It also possesses a nutritional value **[5]**.

Highest production of pepper occurs in India, China, Turkey, Mexico, Spain and Nigeria **[4]**. The fruit of pepper contains phytochemicals such as carotenoids, phenolics, flavonoids and otherantioxidants **[6]**. These compounds protect the body by fighting against the free radicals and preventing the oxidation process **[7-11]**. Free radicals initiate reactions which damage the membrane and interrupt the metabolic pathways, thus alteration of platelet function and increase in the mutations in DNA take place **[7-14]**. Advantages of phytochemicals include reducing the risk of various cancers and cardiovascular diseases.

The active compound present in capsicum is called capsaicin. IUPAC name of capsaicinis 8methyl-N-vanillyl-trans-6-nonenamide. Capsaicin is a vanilloid belongs to the classproto alkaloid. Alkaloids are the heterogeneous compounds which contain nitrogen, carbon and hydrogen. This capsaicin when absorbed by the body gets metabolised by dehydrogenation, thus forms a specific macrocyclic, diene and imide metabolites.

In present study, the methanolic extract of three different varieties of capsicum ((a) Red chilli (b) Green chilli(c) Capsicum were used for phytochemical analysis. It has also been planned for screening of analgesic activity of these capsicum extracts. Later, it has been planned to correlate the presence of phytochemicals in capsicum extracts with the analgesic activity using mouse models.

Figure 1: a) Red chilli (Capsicum annuum) b) Green chilli (Capsicum annuum) c) Capsicum (*Capsicum annuum*)

MATERIALS AND METHODS

Collection of plant material:

Three different varieties of Capsicum were collected from market (Guntur) of weight 2 kg each. The collected plant was authenticated by a botanist Dr M. Suguna, Kumari Department of Botany, BHHS Girls Junior College, Guntur and the voucher specimens were deposited as shown in (**Figure 2)**. Then they were cut into small pieces and kept for shade drying at room temperature 20-25^{0}C. Later, it was grinded into coarse particle using a mechanical grinder (**Figure 3**). The grinded coarse material of plant was subjected for further methanol extraction.

Figure 2: Vocher specimens of different capsicum varieties deposited in the herbarium

A) Red Chilli powder **B) Green chilli powder** **C) Capsicum powder**

Figure 3: Coarse powder of different capsicum varieties

Preparation of extract:

Three different varieties of capsicum (1. Red chilli, 2. Green chilli,3. Capsicum) were size reduced and the weight of each variety was noted. Extraction process was carried out by weighing 87g of green chilli powder in a 1000 ml beaker and 960 ml methanol as a solvent and macerated for 72h using magnetic stirrer. In the similar manner, 38 g of capsicum was weighed in a 500 ml beaker and 450 ml of methanol was added and then macerated for 72h. The third variety red chilli of weight 46 g was taken and macerated in 450 ml of methanol for 72 h. The 3 extracts were filtered using muslin cloth and then the filtrate was kept for evaporation under Rota-evaporator at 100 rpm for 30 min. Later, the concentrated product obtained from Rota-evaporator was kept in fume hood at room temperature for further evaporation. Weight of the concentrated product was noted. Percentage yield was calculated and recorded accordingly. The extract was sealed in a screw cap container and stored at 4^0C for further analysis **[7]**.

Reagents and chemicals:

Reagents used: Molisch, Benedict's, Barfoed's, Fehling's A & B, Selwinaffo's, Mayer's, Hager's, Wagner's, Biuret, Ninhydrin, and Millon's reagents.

Chemicals used: alpha-naphthol, ethanol, sodium carbonate, sodium citrate, copper (ll) sulphatepentahydrate, ethanoic (acetic) acid, copper (ll) acetate, sodium hydroxide, potassium sodium tartrate, resorcinol, concentrated hydrochloric acid, potassium mercuric iodide, picric acid, iodine,

potassium iodide, nitric acid, strong ammonia, concentrated sulphuric acid, benzene, ether, chloroform, concentrated acetic anhydride, bromine water, acetone, ethanol, mercuric nitrate, ferric chloride, magnesium turnings, lead acetate,10% vanillin and 90%mixture of ethanol.

Test for carbohydrates: A biomolecule which consists of carbon, hydrogen and oxygen **[12]**.

A) Molisch test: This test is mainly used for the determination of carbohydrates present in the sample. Molisch test is named after the Australian botanist Hans Molisch.

Chemicals: alpha- naphthol and ethanol are mainly used for the preparation of Molisch reagent.

Procedure: 2ml of sample is taken and Molisch reagent was added to it. The test tube was inclined and concentrated sulphuric acid was added slowly from the sides of the test tube.

Observation: Violet colour ring at the junction of 2 layers indicates the presence of carbohydrates in the sample.

B) Fehling's test: This test was developed by German chemist Hermann von Fehling in the year 1849.

Fehling's test is mainly used for differentiating reducing and non-reducing sugars. Chemicals: Solution A contains copper sulphate. Solution B contains Potassium hydroxide and sodium potassium tartrate.

Procedure: 2 ml of sample is taken, and 2-3 drops of Fehling's A and B are added to it. Heat for 5 min by placing it in a water bath over the Bunsen burner.

Observation: Reddish brown precipitate indicates the presence of reducing sugars in the sample. All monosaccharides give positive for Fehling's test.

C) Benedict's test: This test determines the presence of aldehydes, ketoses, hemiacetals, alpha hydroxy ketones.

Chemicals: Anhydrous sodium carbonate, sodium citrate, copper (II) sulphatepentahydrate.

Procedure: 2 ml of sample was taken, and 2-3 drops of Benedict's reagent was added to it and boiled for 10 min and observed the colour.

Observation: Depending on the amount of sugar, the preset blue colour changes to green, yellow, and brick red precipitate.

D) Barfoed's test: This test is used for determining the presence of monosaccharides in the sample.

Chemicals: Copper acetate in acetic acid solution.

Procedure: 2 ml of sample was taken and few drops of Barfoed's reagent was added to it and boiled for 30 seconds and then allowed it to cool.

Observation: Brick red precipitate indicates the presence of monosaccharides in the sample.

E) Selwinaffo's test: This chemical test distinguishes between aldoses and ketose sugars

Procedure: 2ml of sample was taken and few drops of Seliwanoff's reagent was added to it and boiled for 10-15 min.

Observation: Presence of cherry red colour within 15 min indicates the presence of ketose sugars; Absence of cherry red- aldose sugars.

Test for Alkaloids:

Alkaloids are a class of naturally occurring organic compounds that mostly contain basic nitrogen atoms. Alkaloids can be distinguished by 3 general tests.

A) Mayer's test: This is the general test for alkaloids. Mayer's reagent is an alkaloidal precipitating reagent. This test was invented by the German Chemist, Julius Robert Von Mayer (1814–1878).

Chemicals: Mixture of mercuric chloride and potassium iodide in distilled water.

Procedure: 2ml of sample was taken in a test tube and Mayer's reagent was added to it. Observation: Cream coloured precipitate indicates the presence of alkaloids in the sample.

B) Hager's test:

Chemicals: Picric acid in distilled water

Procedure: 2ml of sample should be taken, and few drops of Hager's reagent was added to it.

Observation: yellow colour precipitate indicates the presence of alkaloids.

C)Wagner's test:

Chemicals: Mixture of iodine and potassium iodide in distilled water [13].

Procedure: 2ml of sample should be taken in a test tube and Wagner's reagent is added to it.

Observation: alkaloids shows reddish brown precipitate.

Test for Purine derivatives

A) Mureoxide test: This test is used to identify the presence of caffeine and other purine derivatives.

Procedure: 2ml of sample should be taken in a china dish and few drops of potassium chlorate and hydrochloric acid is added to it. Place it over the flame. Heat it until it gets evaporated. Cool it and add ammonia to it.

Observation**:** Pseudoalkaloids like caffeine and other purine derivatives show pink colour.

Test for Glycosides:

A molecule in which sugar is attached to another functional group via glycosidic bond.

A) Keller-killiani test: This test is used to identify cardiac glycosides.

Procedure: 2ml of drug+ 10ml of 70% alcohol was soaked for few minutes and

filtered. To 5ml of filtrate, 10ml of hydrogen peroxide and 0.5ml of strong solution of lead acetate was added. To this mixture 2-3 drops concentrated sulphuric acid was added.

Observation: Appearance of blue colour confirms the presence of deoxy sugars.

B) Borntrager's test: This test is used to screen anthraquinone glycosides. Procedure: The drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene, or ether or chloroform was added and shaken well. The organic layer was separated to which ammonia was added slowly.

Observation: Ammonical layer shows pink colour for anthraquinone glycosides.

Tests for proteins:

Proteins are the macromolecules formed from amino acids.

A) Biuret test: Also called Piotrowski'stest. Biuret test is the general test for proteins.

Chemicals: Hydrated copper sulphate, potassium sodium tartrate, sodium hydroxide.

Procedure: 2ml of sample+ few drops of biuret reagent should be added.

Observation: Pink or purple colour shows the presence of proteins.

B) Ninhydrine test: Used to detect ammonia or primary and secondary amines.

Chemicals: 8 g of Ninhydrine in 100 ml of acetone

Procedure: 2ml of sample+ few drops of Ninhydrine reagent.

Observation: violet colour.

Tests for tannins:

Bromine water test: Bromine water test is a qualitative test, used to identify the alkene or alkane functional groups present in the compound. Procedure: 2ml of sample+ few drops of bromine water solution Observation: Orange red colour.

Tests for flavonoids:

A) Shinoda test: To the sample, few drops of magnesium ribbon and concentrated HCl was added.

Observation: Pink or red colour changes to bluish green colour.

B) Ferric chloride test: 2ml of sample+ ferric chloride solution.

Observation: bluish green colour indicates the presence of flavonoids

RESULTS AND DISCUSSION

Percentage yield of methanolic extract of 3 capsicum varieties

Percentage yield of Green chilli = 8.9% w/w

Percentage yield of Capsicum = 10% w/w

Percentage yield of Red chilli= 17.8% w/w

Phytochemical screening of methanolic extract of 3 capsicum varieties

Table 1: Tests for Carbohydrates

S.No.	Tests for Carbohydrates	Green chilli methanolic extract	Red chillimethanolicextract	Capsicummethanolic extract
1.	Molisch test	positive	positive	positive
2.	Benedict's test	positive	positive	positive
3.	Barfoed's tests	positive	positive	positive
4.	Seliwanoff's test	positive	positive	positive
5.	Fehling's test	positive	positive	positive

Table 2: Tests for Alkaloids

S.No.	Tests for Alkaloids	Green chilli extract	Red chilli extract	Capsicum extract
1.	Mayer's reagent	Positive	Positive	Positive
2.	Wagner's reagent	Positive	Positive	Positive
3.	Hager's reagent	Positive	Positive	Positive

Table 3: Tests for Glycosides

S. No.	Tests for Glycosides	Green chilli extract	Red chilli extract	Capsicum extract
1.	Keller-killiani test	Negative	Negative	Negative
2.	Borntrager's test	Negative	Negative	Negative
3.	Saponins test	Negative	Negative	Negative

Table 4: Tests for Flavonoids

S. No.	Tests for flavonoids	Green chilli extract	Red chilli extract	Capsicum extract
1.	Ferric chloride test	Negative	Negative	Negative
2.	Lead acetate test	Negative	Negative	Negative

Table 5: Tests for Proteins

S. No.	Tests for Proteins	Green chilli extract	Red chilli extract	Capsicum extract
1.	Biuret test	negative	negative	negative
2.	Ninhydrine test	negative	negative	negative

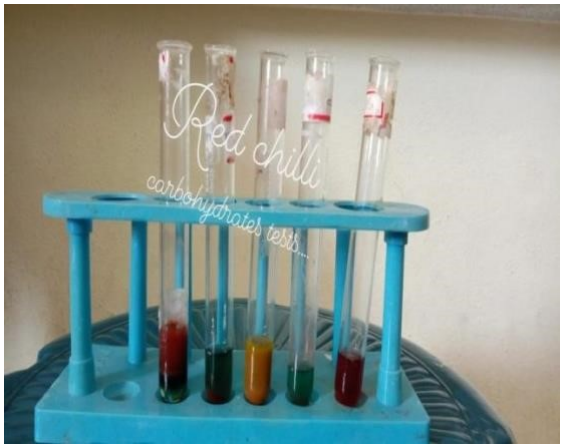
Table 6: Tests for Tannins

S. No.	Tests for tannins	Green chilli extract	Red chilli extract	Capsicum extract
1.	Bromine water test	positive	positive	positive

CARBOHYDRATES TESTS

Molisch test:

Greenchilli **Capsicum**



Red chilli

Fehling's test:

Green chilli **Capsicum** **Red chilli**

Benedict's test:

Green chilli **capsicum** **red chilli**

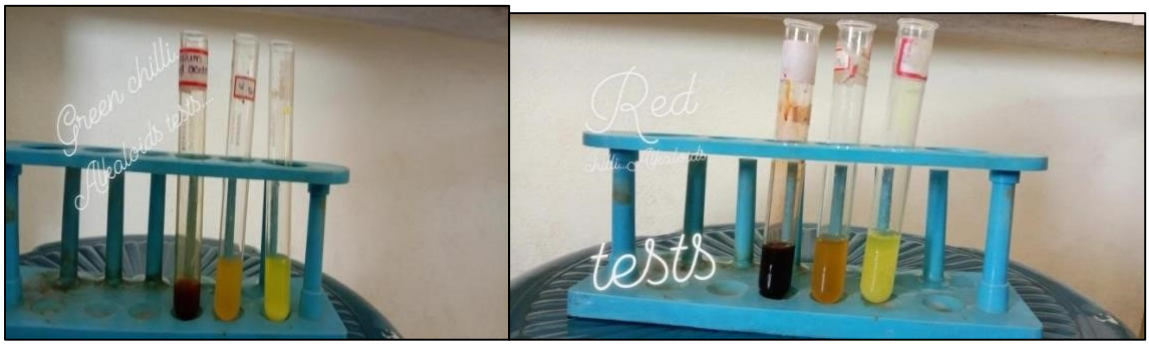
Barfoed's test:

Green chilli **red chilli** **capsicum**

Seliwanoff's test:

Green chilli **red chilli** **capsicum**

Alkaloids tests:



Green chilli **Red chilli**

Capsicum

Mayer's test:

Green chilli **red chilli** **capsicum**

Hager's test:

Greenchilli **red chilli** **capsicum**

Wagner's Test:

Green chilli **red chilli** **capsicum**

Glycosides test:

Green chilli **red chilli** **capsicum**

Flavonoids tests:

Lead acetate test **Ferric chloride test**

Tannins test:

Bromine water tests

Figure 4: Phytochemical study results of methanolic extract of capsicum fruits

SUMMARY

By comparing the phytochemical analysis, the methanolic extract of three different varieties of capsicum (i.e.) red chilli, green chilli and capsicum (bell pepper) showed positive results forcarbohydrates, alkaloids and tannins; and negative result for proteins, flavonoids, and glycosides.

CONCLUSION

Based on the present study results, we concluded that the 3 different varieties of capsicum show similar photochemical results that mean they contain same bioactive compounds.

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