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Phytochemical screening and total phenolic content of Kalonji and Ajwain seeds

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Abstract
Spices are building block of flavour in food. Their primary functions are to provide aroma, texture and colour to food. In addition they also act as preservative and provide nutritional, and health benefits. Nigella Sativa (black caraway also known as Nigella or Kalonji), often called black cumin, is an annual flowering plant in a family Ranunculaceae. Ajwain (Trachyspermum Ammi (L) is one of semi-parasitic plants belonging to the Appiaceae family. A large no of spices are claimed to possess the antioxidant and antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. The purpose of the study was to determine the content of secondary metabolites in kalonji and ajwain extract through phytochemical screening and total phenolic content. For this methanolic extract of kalonji and ajwain was prepared by soxhlet extraction method. After extraction, the extract was taken in beaker and kept on hot plate and heated at 30-40 ºC till all the solvent got evaporated. Dried extract was used for the further study. The highest total phenolic content was found in kalonji i.e. 3.028mg GAE/mg as compare to ajwain that is 2.164mg GAE/mg. Whereas terpenoids was absent in methanolic extract (kalonji) and steroids was absent in methanolic extract (ajwain). The aqueous extract of kalonji and ajwain was prepared by boiling with distilled water. The extract was taken in beaker and kept on hot plate and heated at 60 ºC till all the solvent got evaporated. Dried extract was used for the further study.
The weight of the dried extract was found in kalonji that is 8.24gm as compare to ajwain that is 6.78gm. Whereas tannins are present in (kalonji) aq extract and absent in (ajwain) aq extract.

Keywords: Kalonji, Ajwain, photochemical screening, total phenolic content

1. Introduction
The seeds and plants are a treasure house of potential drugs and in the recent years there has been increasing awareness about the importance of medicinal plants and their seeds. Plant-derived substances have recently become of great interest owing to their versatile applications. [1]. Spices are natural compounds derived from many parts of plants: seed, flowers, fruits, leaves, bark, roots and rhizomes etc. Spices not only used for dietary purposes like aroma, colour, taste, flavour and preservations of foods but also used as a medicine in traditional system of medicine. Spices have their own unique aroma and flavour which derived from photochemical compounds [2]. Dried kalonji and ajwain is rich in polyphenolic compound, a large class of plant based compound through to impart antioxidant properties. In 2010 Scientists at Miguel Hernandez University in Spain reported that ajwain rank highest as a natural antioxidant due to its phenol content and demonstrated ability to inhibit several damaging oxidative processes. Kalonji is good source of iron and keep immune system healthy. Water boiled with kalonji seed is good for coping with dysentery. One study found that kalonji was protective against memory lost and the damaging effect of stress on the body [3]. Another study evaluated the antioxidant content of kalonji and found it more effective than other antioxidant content that it might have a role in fighting cancer. Ajwain is used as carminative, to increase hydrochloric acid in stomach and to improve peristalsis. Ajwain IS said to be nature anthelmintic, Balch, Phyllis and belch, James prescription for nutritional healing [4]. Ajwain oil is beneficial for coping with tooth ache and sore gum. It is also beneficial remedy for chest pain, fever, digestive problem, cough and cold. In addition, clove oil is used in preparation of some tooth paste and clove vaccine solution, which is local anaesthetic use

in oral ulceration and inflammation [5] Present study is undertaken to find out phenolic content and phytochemical study of kalonji and ajwain.

2. Material and Methods

The kalonji and ajwain were collected from the Akola market.

2.1 Preparation of seeds extract

The seeds of kalonji were washed under running tap water. It was then dried under shade and ground into coarse powder in the electronic grinder.

2.2 Solvent extraction

2.2.1 Preparation of methanolic extract (Kalonji)

Kalonji seeds extract was prepared by Soxhlet extraction method. About 20gm of dried powdered seed was mixed with 100ml methanol and continuous extraction was done for 5-6 hours. The temperature was maintained at 30-40 ºC. The process of extraction was continued till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a conical flask. Methanol is then completely removed using hot plate /vacuum evaporator. The weight was recorded. The extract was kept in freezer at 4 ºC until further use.

2.2.2 Preparation of methanolic extract (Ajwain)

Ajwain seeds extract was prepared by Soxhlet extraction method. About 20gm of dried powdered seed was mixed with 100ml methanol and continuous extraction was done for 5-6 hours. The temperature was maintained at 30-40 ºC. The process of extraction was continued till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a conical flask. Methanol is then completely removed using hot plate /vacuum evaporator. The weight was recorded. The extract was kept in freezer at 4 ºC until further use.

2.2.3 Preparation of aqueous extract (Kalonji)

The aqueous extract was prepared by boiling method. About 20gm of kalonji powered with 100ml of distilled water and continuous boiling at 30min. The ratio used was 1:5 (w/v). The solution was then filtered through filter paper attached to the vaccum pump at 30-40pka. The filtrate was then heated on hot plate with temperature below 60 ºC until 24hours and the weight was recorded.

2.2.4 Preparation of aqueous extract (Ajwain)

The aqueous extract was prepared by boiling method. About 20gm of Ajwain powered with 100ml of distilled water and continuous boiling at 30min. The ratio used was 1:5 (w/v). The solution was then filtered through filter paper attached to the vaccum pump at 30-40pka. The filtrate was then heated on hot plate with temperature below 60 ºC until 24 hours and the weight was recorded.

2.3 Phytochemical screening

Chemical tests were carried out to evaluate the presence of the phytochemical Alkaloids, Flavanoids, Terpenoids, Saponins, Tannins and Sterols in the selected species, using standard procedures as described by Sofowora (1993),Trease and Evans(1983).

2.4 Test for alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.5 Test for flavanoids

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids. Another test was performed by mixing crude extract with few fragments of magnesium ribbon. Then concentrated HCl was added drop wise. The development of pink scarlet coloration after few minutes indicated the presence of flavanoids.

2.6 Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and shaken vigorously it was then left to stand for10 minutes and the result was noted. No thick persistent froth was observed. This indicated the absence of saponins.

2.7 Test for proteins

Crude extract when mixed with 2 ml of Million's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of proteins.

2.8 Test for steroids

Crude extract was mixed with 2ml of chloroform and concentrated H2SO4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

2.9 Test for phenol compounds and tannins

Crude extract was mixed with 2ml of 2% solution of FeCl3. A blue-green coloration indicated the presence of phenol compounds and tannins.

2.10 Test for fixed oils and fats

A few drops of 0.5 N alcoholic KOH were added to a small quantity of crude extract along with a drop of phenolphthalein. The mixture was heated on water bath for 2hrs. Partial neutralization of alkali indicated the presence of fixed oils and fats.

2.11 Total phenol Content

The determination of TPC was conducted according to the methods described by Mohld Ilham *et al* (2008). The aqueous and methanolic extracts (100mg) were weight separately and dissolved in 1ml of 1% hydrochloric acid in methanol solvent (v/v). The extract were then centrifuged at 6000rpm for 60min.One (100 µL) of each supernatant were pipette into a bottle after which 750µL) of follin-ciocalteau reagent (10*dilution) was added. The solution were left to stand at room temperature (25 ºC) for 5min.After that 750 µL of sodium bicarbonate (60mg/ml)was mixed into the solution and left to react in dark for 90min. Distilled water was used as a blank in the analysis. The absorbance of the sample was read at 725nm by using visible-spectrophotometer. The TPC was calculated by comparing the absorbance with the tannic acid calibration curve according to the formula.

TPC (Mg/g) =C ×v/g
Where,
C = Concentration of the tannic acid equivalent from standard curve
V = Volume of the extract used (ml)
g = Weight of extract (gm)

3. Results and Discussion

3.1 Phytochemical screening of kalonji and ajwain: The phytochemical screening carried out on methanolic extract of kalonji was absent in terpenoids and the methanolic extract of ajwain was absent in sterols. The weight of the dried extract was found in kalonji (methanolic extract) that is 3.028gm as compare to ajwain that is 2.164gm. Whereas tannins were present in (kalonji) aq extract and absent in (ajwain) aq extract. The weight of the dried extract was found in aq extract of kalonji that is 8.24gm as compared to ajwain that is 6.78gm.

Table 1: Phytochemical screening of kalonji and ajwain

S.no	Constituent	Colouration	Aq extract of kalonji	Methanolic extract of kalonji
1	Alkaloids by-			
a)	Mayers reagent	Dark brown	+	+
b)	Wagner's reagent	Dark brown	+	+
2	Flavanoids	Yellow	+	+
3	Terpenoids	Reddish brown colour	+	-
4	Saponins	No formation of foam	+	+
5	Sterols	Red ppt	+	+
6	Tannins	Bluish green	+	+
7	Protein by-			
a)	Xanthoprothetic test	Yellow colour	+	+
b)	Ninhydrin test	Blue colour	+	+
8	Phenols	Blue colour	+	+

Table 2: Phytochemical screening of kalonji and ajwain

S.no	Constituent	Colouration	Aq extract of Ajwain	Methanolic extract of Ajwain
1	Alkaloids by-			
a)	Mayer's reagent	Dark brown	+	+
b)	Wagner's reagent	Dark brown	+	+
2	Flavanoids	Yellow	+	+
3	Terpenoids	Reddish brown colour	+	-
4	Saponins	No formation of foam	+	+
5	Sterols	Red ppt	+	-
6	Tannins	Bluish green	-	+
7	Protein by-		+	
a)	Xanthoprothetic test	Yellow colour	+	+
b)	Ninhydrin test	Blue colour	+	+
8	Phenols	Blue colour	+	+

3.2 Total phenolic content of kalonji and ajwain

The weight of the dried extract was found in kalonji (methanolic extract) that is 3.028gm as compared to ajwain that is 2.164gm. The weight of the dried extract was found in aq extract of kalonji that is 8.24gm as compared to ajwain that is 6.78gm.The total phenolic content (TPC) was determined in comparision with standard tannic acid and the results were expressed in term of mgGAE/dry sample. The TPC of kalonji (methanolic extract) was found to be 0.3963mgGAE/100ml as compared to ajwain extract was found to be 0.5314mgGAE/100ml.The TPC of kalonji (aq extract) was found to be 0.0485mgGAE/100ml as compared to ajwain extract was found to be 0.0294mgGAE/100ml.The TPC was calculated by comparing the absorbance with the tannic acid calibration curve.

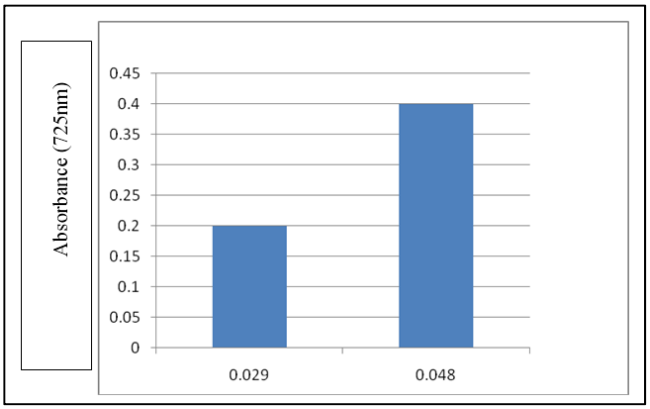
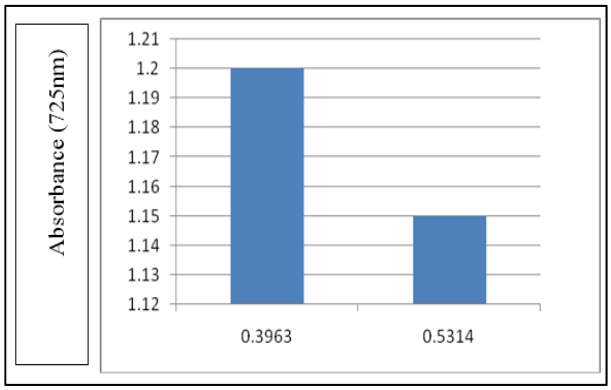


Fig 1: The graph between total phenolic content of Aq extract (µg/g) and the absorbance (725nm).



Total phenolic content of methanolic extract

Fig 2: The graph between total phenolic content of methanolic extract (µg/g) and the absorbance (725nm).

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