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Microwave assisted extraction, fractionation and total phenolic and flavanoid estimation of *Annona muricata* leaves

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ABSTRACT

The present study focuses on the microwave assisted extraction of *Annona muricata* leaves in ethanol. The extract obtained was subjected to column chromatography and the fractionation was done to get 10 different fractions. Preliminary phytochemical screening was done and the fractions were subjected to total phenolic and flavanoid content estimation. The crude extract contained 490 mg GAE/100g of phenolic content and among the fractions AM8 contained the maximum amount of phenolic content that is 360 mg GAE/100g. The flavanoid content present in the crude extract of the *Annona muricata* was found to be 127mg QE/100g Among all the tested fractions, the fraction AM8 showed more flavanoids that is 97.5 mg QE/100g and the study is to be extended to find out the components present in the active fraction.

INTRODUCTION

Flavanoids are the largest group of naturally occurring phenolic compounds. They occur both in free state and as glycosides. Flavones and flavonols are widely distributed. They have a wide variety of biological activities. Flavanoids act as antioxidants acting against degeneration, cardiovascular disorders and also inhibit tumor development in animal models. Some are acting as heart stimulant [1]. They scavenge the free radicals such as superoxide and hydroxyl radicals.

Annona muricata belongs to the family Annonaceae. It is also known as 'Graviola'. For the extraction of secondary metabolites from plants microwave assisted extraction is playing an important role. Microwave Assisted Extraction (MAE) can be used for the extraction of functional group.Here microwave energy is absorbed by the molecules. Strong penetrating force, high selectivity, high heating ability, less extraction time, reduced solvent consumption and energy requirements are the main advantages of this method [2,3].The application of microwave assisted extraction of secondary metabolites from plants also have been published [4,5].

MATERIALS AND METHODS

Plant material

The plant *Annona muricata* belonging to the family Annonaceae was collected from Neyyattinkara, Thiruvananthapuram District, Kerala. It was authentified by Dr. Jomy Augustine, Department of Botany, St. Thomas College, Pala, Kottayam, Kerala. A specimen with voucher no.1501 was kept in the department for further reference.

Extraction and fractionation

The leaves of *Annona muricata* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieveNo.42 and stored in an airtight container for further use. The dried material was extracted in ethanol by scientific microwave oven. The extraction time was 5 minutes and the power used was 850W. About 30ml of ethanol was used as the solvent. The extract was made solvent free by distillation process and the resulting semisolid mass was vacuum dried to yield a solid residue. The experiment was repeated to get 20g of the extract.

Isolation of the compounds through column chromatography started by using 20g of the extract. A slurry of the extract was prepared in ethanol and the extract was uniformly packed over dry

silica gel (mesh size 230-400, 20g). Petroleum ether, Petroleum ether: chloroform (4:3, 3:2, 2:3 and 1:4), Chloroform, Chloroform: Ethanol (4:3, 3:2, 2:3 and 1:4) such different solvents in different ratios were used as the mobile phase. Similar small fractions were collected in small test tubes. TLC (silica gel F254) of all individual fractions were developed. It was then viewed under UV chamber. Based on the TLC results similar fractions were pooled. The fractions were dried in rotavapor under reduced pressure at a temperature of about 40+_5º.Such 10 combined fractions were collected. Preliminary phytochemical screening was done by using the extract and fractions by standard methods [6].

Determination of total phenolic content

Folin-ciocalteau reagent was used for the determination of total phenolic content and the standard used was gallic acid. The reagent (5ml) was mixed with 1ml of gallic acid at different concentrations and after 3 minutes 4ml of 2% sodium carbonate was added to each solution. After 30 minutes blue colour was developed. It was then read at 760nm. Different concentrations of the sample extract were treated in the similar manner. The test was repeated thrice. The concentration of the total phenol was expressed as mg/g of dry extract.

Determination of total flavanoids

Total flavanoid content was determined by aluminium chloride colorimetric assay [7]. Distilled water (4ml) was taken in 10ml of volumetric flask and an aliquot quantity of extract (1ml) or standard solution of quercetin (25-150µg/ml) was added to it. Sodium nitrite 5% (0.3ml) was added and after 5min,0.3ml of 10% aluminium chloride was added. After 5 min, 2ml of 1M sodium hydroxide was added and the volume was made up to 10ml with distilled water. The solution was mixed well and the absorbance was measured against the blank at 510nm. The total flavanoid content was expressed as mg quercetin equivalents (QE).

RESULTS

In the present study the extraction of the plant *Annona muricata* was carried out by microwave assisted method and the fractionation was done by column chromatography using petroleum ether, chloroform and ethanol at different ratios (Table 1). At different concentrations of gallic acid absorbance was calculated (Table: 2) and the standard graph was plotted (Fig.1).

Total phenolic content present in the extract and the fractions were calculated from the standard graph of the gallic acid. The results are shown in table (Table 3). It was found that the fraction AM 8 contained more amounts of the phenolic constituents. At different concentrations of quercetin absorbance were calculated (Table 4) and the calibration curve of quercetin was plotted (Fig.2). The total flavanoid content of the extract and the fractions were calculated from the standard graph of quercetin and was expressed as mg quercetin equivalents (QE). The results are shown in the table (Table 5).

DISCUSSION

A wealth of studies had been conducted on *Annona muricata* leaves due to various activities. But the promising activity is the

Table 1. : Different Fractions of Annona muricata leaves ethanol extract

Fractions	Colour and consistency	Percentage yield (%W/W)
Petroleum ether (AM1)	Orange dry mass	3.00
Petroleum ether:Chloroform 4:1 (AM2)	Green sticky mass	2.05
Petroleum ether:Chloroform 3:2 (AM3)	Dark green sticky mass	3.30
Petroleum ether:Chloroform 2:3 (AM4)	Brownish green sticky mass	3.15
Petroleum ether:Chloroform 1:4 (AM5)	Brownish green sticky mass	3.45
Chloroform (AM6)	Brown dry mass	3.10
Chloroform:Ethanol 4:1 (AM7)	Brown sticky mass	2.55
Chloroform:Ethanol 3:2 (AM8)	Greenish brown sticky mass	6.00
Chloroform:Ethanol 2:3 (AM9)	Greenish brown sticky mass	0.95
Chloroform:Ethanol 1:4 (AM10)	Brown sticky mass	3.20

Table 2. : Absorbance obtained at different concentrations of gallic acid

Sl.NO	Concentration of gallic acid (µg/ml)	Absorbance
1	100	0.842+_0.0002
2	200	1.600+_0.0002
3	300	2.116+_0.0002
4	400	2.913+_0.0002
5	500	3.600+_0.0002

Table 3. : Total phenolic content present in Annona muricata ethanol extract and fractions

Sample	Absorbance	Total phenolics mg GAE/100g
Extract	3.52	490
AM1	0.856	100
AM2	0.996	110
AM3	2.112	300
AM4	1.592	200
AM5	1.521	190
AM6	1.336	170
AM7	1.006	120
AM8	2.524	360
AM9	2.002	290
AM10	1.952	270

Table 4. : Absorbance obtained at different concentrations of quercetin

Sl.NO.	Concentration of quercetin (µg/ml)	Mean absorbance
1	25	0.285+_0.0002
2	50	0.432+_0.0002
3	75	0.612+_0.0002
4	100	0.798+_0.0002
5	125	0.992+_0.0002
6	150	1.122+_0.0002

Table 5. : Total flavanoid content present in Annona muricata ethanol extract and fractions

Sample	Absorbance	Total flavanoids (mg QE/100g)
Extract	1.120+_0.0002	127
AM1	0.112+_0.0002	2.5
AM2	0.200+_0.0002	15
AM3	0.661+_0.0002	80
AM4	0.680+_0.0002	85
AM5	0.675+_0.0002	82.5
AM6	0.326+_0.0002	32.5
AM7	0.366+_0.0002	37.5
AM8	0.775+_0.0002	97.5
AM9	0.314+_0.0002	30
AM10	0.412+_0.0002	45

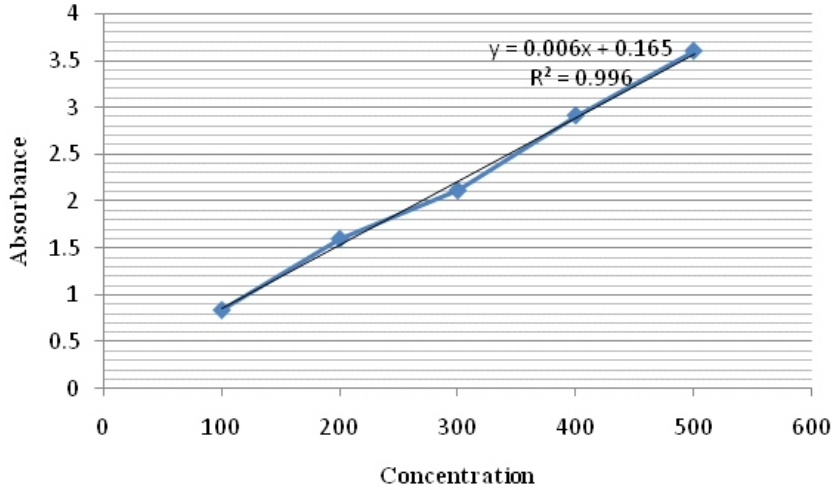

Table 1. : Category of drugs prescribed in geriatric patients

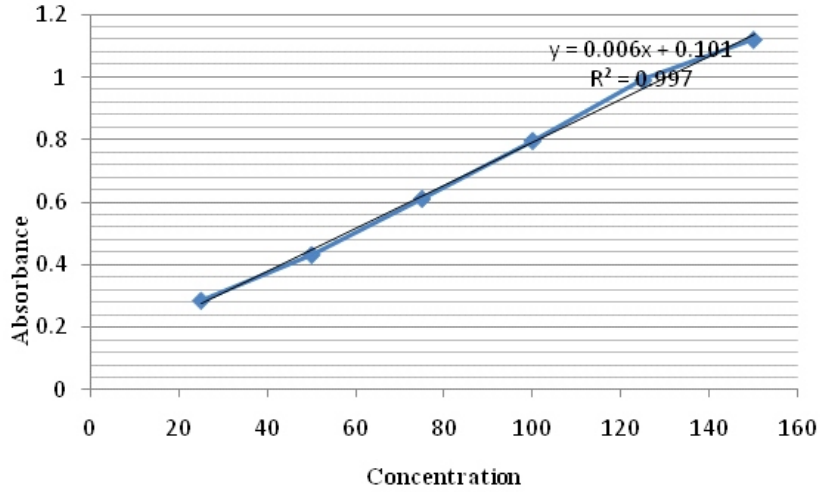

Table 2. : Calibration curve of quercetin

anticancer effect. But most of the activities are concentrating on the extracts obtained from it. So the present study focused by using the fractions obtained from the extracts.

The microwave assisted method of extraction used in the present study is a widely accepted method for the removal of active components from the crude drug. The method helped to save time and the amount of solvents used. The literatures describe as the presence of phenolic content is the important criteria which attributes for many activities of the plant materials.

It is commonly known that the phenolic constituents and the flavanoids are related to various biological activities. They in turn affects the human health. So the present study was focused on the determination of total phenolic and flavanoid content. Since the ethanol extract and the fractions of *Annona muricata* are showing more amount of phenolic and flavanoid, this may show various biological activities including the anticancer activity.

CONCLUSION

Many plants are showing various pharmacological activities due to the presence of phenolic and flavanoid contents. *Annona muricata* is the plant coming under Annonaceae family having wide variety of biological activities. The microwave assisted ethanol extract of *Annona muricata* leaves are showing the presence of phenolics and flavanoids. From this study it is clear that the fraction 8 of *Annona muricata* ethanol extract is containing more amount of phenolics and flavanoids. So this fraction may show more antioxidant and anticancer activities. This fraction is to be taken for further study for the isolation of active constituents.

Conflicts of interest

Authors do not have any financial conflict of interests.

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