INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PREPARATION OF A COMPOSITE SEED POWDER, PRELIMINARY PHARMACOGNOSTIC STUDIES AND PRODUCT DEVELOPMENT USING THE COMPOSITE SEED POWDER

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Article Received on: 03/11/17 Approved for publication: 22/11/17

DOI: 10.7897/2230-8407.0811230

ABSTRACT

The objective of the present study was to prepare a composite seed powder made up of seeds from plants known for their medicinal properties and to study the pharmacognostic activity of this powder. Subsequently, four sweet and three spicy food products (Indian preparation) were developed with this powder. *In-vitro* anti-arthritic activity of the hexane and ethyl acetate extracts was assessed by inhibition of protein denaturation method. The extracts of the seed powder were evaluated for free radical scavenging activity using DPPH. The *in-vitro* antimicrobial activity was determined using cup-plate method against two each gram positive and gram negative bacteria and fungi. The seed powder was used in the preparation of seven simple snack items which were evaluated on a five point-Hedonic Scale by a panel for taste, flavor, appearance texture and overall acceptance. The nutritive values of each product were computed. The hexane extract showed greater *in-vitro* anti-arthritic and anti-oxidant activity than that of the ethyl acetate extract. The ethyl acetate showed higher antibacterial activity against the test bacteria. All the products developed with the composite seed powder were well accepted and appreciated for their distinct flavor, good taste, appealing appearance and unmatched texture. The composite seed powder is rich in phytochemicals, hence exhibiting anti-arthritic, antioxidant and antimicrobial activity. The powder is also palatable and fit for human consumption in small amounts, as it is made from the seeds of edible fruits and vegetables.

Keywords: Anti-arthritic activity, anti-oxidant activity, anti-microbial activity, product development, sensory evaluation

INTRODUCTION

India is known to be blessed with an innumerable number of medicinal plants. Many of these medicinal plants are toxic and unsafe for human consumption. However, there are other plants with known medicinal properties that are non-toxic and edible, some forming an important part of the diet. Five such plants, namely, Papaya[1,2,3], Pomegranate[4,5,6], Watermelon[7,8,9], Bitter gourd[10,11,12] and Capsicum[13,14,15] known for their medicinal properties, which are also edible, were selected. The study aimed at scientifically evaluating the *in vitro* anti-arthritic activity, antioxidant activity and antimicrobial activity of the seeds of the selected plants. In addition, seven traditional Indian dishes were prepared incorporating the seed powder that was obtained from these medicinal plants. Sensory tests were performed on these dishes to assess the various parameters taste, flavor, appearance, texture and overall acceptance.

MATERIAL AND METHODS

After literature survey it was observed that the seeds of fruits-Papaya, Pomegranate & Watermelon and vegetables-Bitter gourd & Capsicum are rich in various phytochemicals and nutritive elements; contributing to the overall health of the individual. Hence the seeds of these selected plants were taken, shade dried and made to a fine powder. This powder, henceforth referred to as 'seed powder' was used for the following studies.

The fruits/ vegetables were bought from Purna Market, Visakhapatanam, Andhra Pradesh, India, during January 2016. The seeds were carefully removed and dried in the shade for two weeks, following which they were made to a fine powder by grinding in a mixer grinder.

Extraction Process

The seed powder was separately extracted with hexane and ethyl acetate by the process of Maceration[16]. The extracts thus obtained were concentrated under vacuum at a temperature of 54°C by using a rotary evaporator. The oil obtained was quantified and then dissolved in a suitable solvent and stored for further use in a refrigerator. The amount of the fractions obtained from the seeds powder is as follows: Ethyl acetate > Hexane.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis was carried out by standard protocols on the extracts to detect various phytoconstituents present[17].

***In-vitro* Anti-arthritic Activity**

Anti- denaturation study is performed by using bovine serum albumin (BSA). When BSA is heated it undergoes denaturation and expresses antigens associated with type- III hypersensitivity reaction and that is related to diseases such as serum sickness, rheumatoid arthritis and Systemic lupus erythematosus [18].

***In-vitro* Anti-oxidant Activity**

DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue) in the presence of a hydrogen donating antioxidant converting them to the radical form of yellow colored diphenyl–picrylhydrazine[19].

***In-vitro* Anti-microbial Activity**

The cup-plate method is based on measuring the diameter of zone of inhibition of microbial growth surrounding the cups containing various dilutions of extracts[20,21].

Product Development

Seven different snacks were prepared, adding the composite seed powder in each item. The food products developed were then subjected to sensory evaluation.

Organoleptic evaluation of the developed products

Sensory evaluation/ organoleptic evaluation of the developed products was done by 11 selected panel members. All the products were evaluatedon the basis of various parameters like taste, appearance, texture, flavor and overall acceptability. Each parameter was evaluated on a five-pointhedonic scale[22] by various scores, which are as follows:
Scores:
1. Not acceptable
2. Average/ satisfactory
3. Good
4. Very Good
5. Excellent

Computation of Nutritive value of the developed products

Nutritive values were computed for all the recipes based on the nutrient information database of raw ingredients[23].

RESULTS AND DISCUSSION
Preliminary Phytochemical Analysis

The hexane extract that was obtained was a concentrated oil. The ethyl acetate extract was also obtained in a similar form, as oil, but not as viscous as the hexane extract. Phytochemical analysis of the two extracts revealed the presence of various phytoconstituents in both the seed extracts. The hexane extract gave positive results for the tests for phytosterols, saponins, flavonoids, tannins, carbohydrates, alkaloids, oils, quinones and phenols, indicating the presence of all these phytochemicals. High coloration was observed in the tests for phytosterols and quinines indicating the presence of more amounts of phytosterols and quinones. Triterpenes and glycosides were absent in the hexane extract. The ethyl acetate extract also showed the presence of several phytochemicals such as phytosterols, saponins, flavonoids, tannins, alkaloids, oils, quinones and phenols. Triterpenes, glycosides and carbohydrates were absent in the ethyl acetate extract. Phytosterols, saponins and quinones gave excellent reactions as indicated by the double/triple +. The results are detailed in Table 5.

***In-vitro* Anti-arthritic Activity**

The *in-vitro* anti-arthritic activity of the hexane and ethyl acetate fractions of the composite seed power were assessed. Both the fractions showed a concentration-dependent activity. The results are shown in Fig. 3. The hexane extract showed an excellent anti-arthritic capacity; higher than that of the ethyl acetate extract. The highest activity of the hexane extract was observed at the highest concentration; 1000µl, while the ethyl acetate extract showed the highest activity at the lowest concentration; 250µl. The activity of the hexane extract was almost the same, at all concentrations the percent inhibition of albumin denaturation was above 90%. As the concentration of the extract increased, there was a slight increase in the percent inhibition. The activity at 750 & 1000µl was almost the same.

The anti-arthritic activity of the ethyl acetate extract showed a downward trend; as the concentration of the extract increased, the percent inhibition of protein denaturation decreased. The highest activity was observed at the lowest concentration; 250µl. The activity gradually decreased till the concentration of 1000µl. The percent inhibition of the ethyl acetate extract was much lower than that of the hexane extract.

***In-vitro* Anti-oxidant Activity**

The hexane and ethyl acetate extracts of the seed powder were found to possess concentration-dependent scavenging activity on DPPH radicals and the results are given Fig. 4. Among the two extracts, the hexane extract showed more significant antioxidant activity. Both the extracts exhibited higher DPPH radical scavenging potential al the lowest concentration; 250µl. At the two higher concentrations; 750 & 1000µl, no activity was observed both for the hexane and ethyl acetate extract.

***In-vitro* Antimicrobial Activity**

The antibacterial activity of the hexane and ethyl acetate extracts was assessed by cup-plate method. Two gram-positive and two gram-negative bacteria were used for the study. Among the two extracts, the ethyl acetate showed higher antibacterial activity against the test bacteria. The detailed results are given in Table 6.

The hexane extract was potent only against the gram-positive bacteria *S. aureus.* The activity was however less than that of the standard antibiotic, Rifampicin. No activity was observed against the other gram-positive bacteria under study; *B. subtilis* or the gram-negative bacteria under study; *K. pneumoniae* or *P. aeroginosa.*

The ethyl acetate extract however showed better activity than the hexane extract. It was potent at all concentrations against both the gram-positive and gram-negative bacteria used in the study. Among gram-positive and gram-negative bacteria, the ethyl acetate extract was more potent against the gram-negative bacteria. Highest activity was observed against *K. pneumoniae* at a concentration of 100µl, higher than that of the standard; Rifampicin. Highest activity against the other gram-negative bacteria under study was observed at a concentration of 200µl, the activity being very slightly less than that of Rifampicin. The activity of the ethyl acetate extract against the gram-positive bacteria was also fairly good.

TABLE 1: SEEDS USED FOR PREPARATION OF SEED POWDER

S. No.	Plant Name	Scientific Name	Amount Taken
1	Papaya	*Carica papaya*	Seeds of 1 ripe fruit
2	Pomegranate	*Punicagrantum*	Seeds of 4 ripe fruits
3	Watermelon	*Citrullus lantanus*	200 gm
4	Bitter gourd	*Momordica charantia*	Seeds of 10 unripe vegetables
5	Capsicum	*Capsicum annum*	Seeds of 4 unripe vegetables

Figure 1: Seeds Collected

Figure 2: Composite Seed Powder

TABLE 2: DETAILS OF EXTRACTION

Plant Material	Solvent Used	Volume of the Solvent (ml)	Volume of the Extract Obtained (ml)
Seed powder	Hexane	100	11
Seed powder	Ethyl acetate	100	19

TABLE 3: TEST ORGANISMS USED TO DETERMINE ANTI-MICROBIAL ACTIVITY OF COMPOSITE SEED POWDER

Gram Positive Bacteria	Gram Negative Bacteria	Fungi
Staphylococcus aureus (NCIM No.2127)	*Pseudomonas aeroginosa* (NCIM No.2000)	*Aspergillus niger* (NCIM No. 839)
Bacillus subtilis (NCIM NO. 2063	*Klebsiella pneumoniae* (NCIM no.2957)	*Aspergillus flavus* (NCIM No. 1028)

TABLE 4: DETAILS OF SNACKS PREPARED

Sweet			Spicy		
S. No.	**Dish Name**	**Amt. of Seed Powder (in g)**	**S. No.**	**Dish Name**	**Amt. of Seed Powder (in g)**
1	Biscuits	20	**1**	Onion Pakoda	10
2	Ragi Porridge	10	**2**	Veg. corn soup	10
3	Rawa Keseri	30	**3**	Veg. Upma	20
4	Semiya	20			

TABLE 5: PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemicals	Seeds powder	
	Hexane Extract	**Ethyl acetate Extract**
Phytosterols	+ +	+ + +
Triterpenes	-	-
Glycosides	-	-
Saponins	+ +	+ +
Flavonoids	+	+
Tannins	+	+
Carbohydrates	+	-
Alkaloids	+	+
Oils	+ + +	+ + +
Quinones	+ + +	+ +
Phenols	+	+
+ = Present, - = Absent		

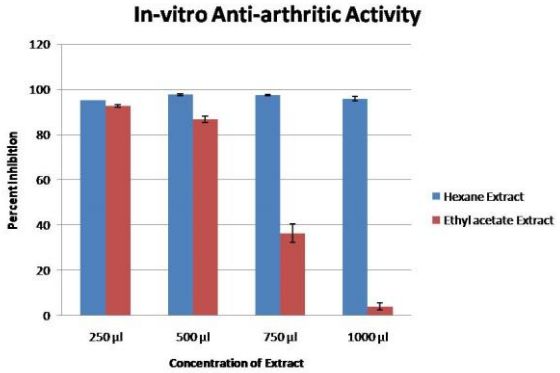

Figure 3: Anti-arthritic activity extracts of Composite Seed Powder

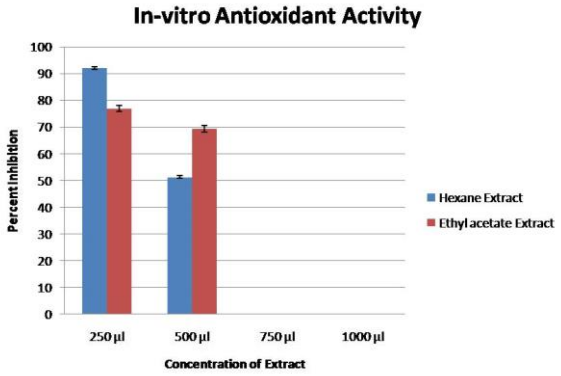

Figure 4: Concentration dependent percent inhibition of DPPH radical by different extracts of the composite seed powder in *In-vitro* studies

TABLE 6: ANTIBACTERIAL ACTIVITY OF THE TWO EXTRACTS OF THE COMPOSITE SEED POWDER

Concentrations (µl)	Zone of inhibition							
	Hexane				Ethyl acetate			
	Sa	*Bs*	*Kp*	*Pa*	*Sa*	*Bs*	*Kp*	*Pa*
50	10	_	_	_	18	16	16	19
100	14	_	_	_	18	18	26	21
150	14	_	_	_	19	19	22	23
200	17	_	_	_	20	22	20	25
Rifampicin	21	22	22	18	24	24	22	22

TABLE 7: ANTIFUNGAL ACTIVITY OF COMPOSITE SEED POWDER

Concentrations (µl)	Zone of inhibition			
	Hexane		Ethyl acetate	
	An	*Af*	*An*	*Af*
50	10	-	_	_
100	_	_	10	_
150	_	_	_	_
200	_	_	15	_
Nystatin	19	_	24	_

Figure 5: Product Development- Food Items Prepared

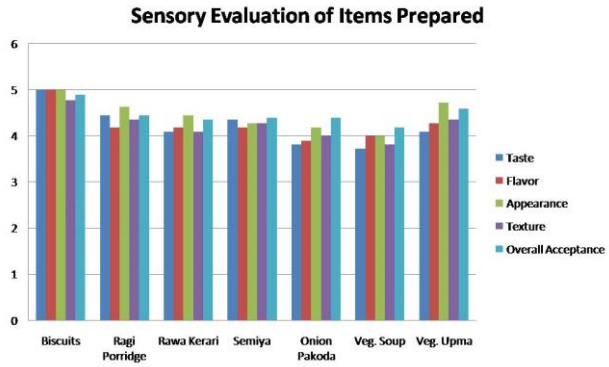

Figure 6: Sensory Evaluation of the Food Items Prepared

TABLE 8: NUTRITIVE VALUE OF INDIVIDUAL SNACK ITEMS DEVELOPED

S. No.	Item Prepared	Nutrients					
		Energy (k. Cal.)	Protein (g)	Carbohydrates (g)	Fat (g)	Calcium (mg)	Iron (mg)
1	Biscuits	999.2	10.36	124.74	51.0	71.2	15.593
2	Ragi Porridge	978.8	25.39	118.0	33.21	1270.0	4.534
3	Rawa Kesari	2072.0	30.55	440.5	18.5	280.0	4.5875
4	Semiya	3164.8	66.06	495.365	78.345	2187.5	7.679
5	Onion Pakoda	1114.08	43.9	159.212	33.434	549.25	12.4686
6	Veg. Soup	87.15	3.39	16.645	0.755	13.0	0.768
7	Veg. Upma	3695.51	29.517	214.224	302.33	122.24	6.4806

The extract was found to be more potent against *S. aureus* when compared to *B. subtilis*, highest activity was observed at the highest concentration; 200µl, but still slightly lower than the standard Rifampicin.

The extracts of the composite seed powder showed low/no *in-vitro* antifungal activity. Neither of the extracts nor the standard used, Nystatin were potent against *A. flavus.* The hexane extract showed antifungal activity against *A. niger* only at a concentration of 50µl, this also being considerably lower than that of Nystatin. The ethyl acetate extract exhibited inhibitory effects against *A. niger* only at two concentrations, 100 & 200µl, both being quite lower than the activity of Nystatin. The results are represented in Table 7.

Product Development and Sensory Evaluation

All seven snack items (four sweet and three spicy) were prepared using simple Indian home recipes. While all the seven items were well accepted, as shown by an overall acceptance score of over 4 for all products, the highest score of 4.89 was given to biscuits showing that the taste of the biscuits were enhanced by the presence of the composite seed powder. An overall acceptance score of 4.59 was given to veg. upma, where it was observed that the seed powder mixed well with the rawa granules to give the final product a good texture and flavor. The remaining snacks prepared obtained overall acceptance scores between 4-4.5.

Among all the snacks prepared, the highest scores for taste (5.0), flavor (5.0), appearance (5.0) and texture (4.78) was given to biscuits by the panel. Veg. upma had the second highest scores for flavor (4.27) and appearance (4.72). Veg. upma and ragi porridge had second highest scores for texture (4.36) and ragi porridge was second for taste (4.45).

The least score for overall acceptance among the seven snacks was observed in the case of veg. soup, the score value being 4.18.

The detailed sensory evaluation and nutritive values of the individual snack items developed with the composite seed powder are described below, in Figure. 6 and Table 8, respectively.

CONCLUSION

It may be stated that this seed powder is like a gold mine for phytochemicals. Both, the hexane and ethyl acetate extract showed the presence of a wide spectrum of phytochemicals like phytosterols, saponins, flavonoids, tannins, alkaloids, oils, quinones and phenols. The hexane extract exhibited high percent of inhibition of denaturation of protein, thus pointing to the fact that the phytoconstituents present in it in all likelihood exhibit anti-arthritic activity. It was noticed that both the hexane and ethyl acetate extract exhibited free radical scavenging activity at lower concentrations, but had absolutely no activity at higher concentrations. The phytochemicals in the ethyl acetate extract were more potent in the control of growth of bacteria, than that of the hexane extract. Little/ no antifungal activity was observed by both the extracts.

All the products developed with the composite seed powder were well accepted and appreciated for their distinct flavor, good taste, appealing appearance and unmatched texture. It was found that the addition of the powder did not adversely impact the taste of either the sweet or the spicy dishes. This is a welcome outcome of the study, as the composite seed powder possesses medicinal properties as established from the pharmacognostic evaluations conducted and it is also rich in nutritive value as computed by standard procedures. If taken in small amounts regularly, it will improve health and wellbeing of the individual. It can be concluded that this powder can be used in the preparation of various dishes to reap the combined medicinal benefits of all these plants as the seeds of these plants are rarely consumed as part of our daily diet (except pomegranate seeds).

ACKNOWLEDGMENTS

The authors are thankful to Andhra University for providing the necessary facilities to carry out this research work.

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Cite this article as:

Angela E. Peter *et al*. Preparation of a composite seed powder, preliminary pharmacognostic studies and product development using the composite seed powder. Int. Res. J. Pharm. 2017;8(11):123-128 http://dx.doi.org/10.7897/2230-8407.0811230

Source of support: Nil, Conflict of interest: None Declared