Research Article

Microwave assisted extraction of phytochemicals from bark of *Cassia occidentalis* L.

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Abstract

Microwave assisted extraction is a modern and new approach for rapid and efficient extraction of plant material which are rich in number of secondary metabolites of prime importance. In present study a comparison was made between conventional method of extraction i.e., Soxhlet extraction, and modern method of extraction i.e., Microwave assisted extraction for the rapid and efficient extraction of *Cassia occidentalis* L. bark. Microwave assisted extraction was proved to be more rapid and more efficient for extraction of bark of *Cassia occidentalis* which yielded 190mg of extract/ 1g of bark powder in 70 seconds of microwave irradiation of 700W as compared to 160mg of extract/30g or 5.33mg of extract per gram of bark powder in 15 hours of continuous heating of the soxhlet extraction. It was also observed that a maximum of 170.87µg equivalent of Gallic acid of phenolics were obtained from extract of Microwave heating (900W) for 30 seconds while 177 µg equivalent of Catechin of flavonoids were obtained from a microwave assisted extract at 70 seconds of 900W of irradiation. In contrast, soxhlet extraction produced only 96.75 µg equivalent of Gallic acid and 120µg equivalent of Catechin after 15 and 10 hours of extraction respectively. Furthermore higher amount of alkaloids were obtained from microwave assisted extraction (3.2 mg eq of Dragondroff reagent). So it was proved that microwave assisted extraction was efficient as well as cost effective in terms of lesser use of energy, time and solvent.

Key words: Microwave assisted extraction; *Cassia occidentalis*; Bark; Phenolics; Flavonoids; Microwaves.

Introduction

Microorganisms with developed drug resistance are a great source of threat for human beings. When antibiotics were discovered, number and intensity of diseases decreased and their use in different fields like food, dentistry, medicine and agriculture increased rapidly. But with the passage of time due to over use and misuse of antibiotics, pathogens evolved with more

resistance towards discovered and formulated antibiotics. Examples of such organisms are strains of *Salmonella enterica*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Xanthomonas* and *Pseudomonas aeruginosa* [1-3].

Due to all above discussed reasons, use of natural bioactive components has become very common and started to increase rapidly

e.g., natural bioactive components of plant extracts. People are now more intended towards herbal medicines. Sources of these natural compounds are usually easily available in surrounding and easy to select on the basis of experience of common man [4]. In this regard a number of plant based drugs are in use e.g., digitoxin from foxglove, Vinblastine from *Catharanthus*, morphine from the opium poppy, quinine from cinchona bark and aspirin from willow bark [5].

Pakistan has a variety of medicinal plants. People living in villages and valleys use indigenous plants of their local areas to cure a number of ailments like asthma, blood pressure, fever, snake bite, diarrhea, jaundice, cough, abdominal pain, eye and kidney infections etc [6, 7].

One of these medicinal plants is *Cassia occidentalis* Linn. (Known as Kasondi) belonging to family Fabaceae and Subfamily Caesalpinioideae and it is very commonly distributed in tropical and warm temperate regions of the world. The plant is 6-8 feet in height. Its roasted seeds are used as coffee giving the idea of its use as functional food (Used as food as well as medicine). Its leaves are also cooked and eaten. Its medicinal value is also valid and reported. Its leaves, seeds and roots are used to cure fever, asthma, tuberculosis, liver problems, kidney problems, menstrual cycle problems etc. particularly its leaves have very prominent antiplasmodial, antimalarial, antimutagenic, antibacterial and anticarcinogenic activity [8, 9].

But to observe all of these activities, extraction is first and essential step to get important phytochemicals from plants. But in our local herbal industry, it's the forbidden step. Extraction in our local industry usually depends upon the common and conventional methods of extraction which produce lesser products and consume more energy. So it is the requirement to optimize some modern

methods to get targeted phytochemicals from selected plants in lesser time frame as well as little use of energy and solvents [10, 11]. One of such modern methods is microwave assisted extraction.

Microwaves are non-ionizing and do not change the structure of targeted molecule. They are considered as safe for extracting natural products. A lot of research work has been done to evaluate the Microwave assisted extraction for extraction of phytoconstituents, but unluckily, there is no work still reported for microwave assisted extraction of *Cassia occidentalis*. So in the present study, Microwave assisted extraction and soxhlet extraction have been done and compared for efficacy for production of extracts, phenolics, flavonoids and alkaloids.

Materials and Methods

The description of plant was confirmed by different flora [12, 13]. Plant material (bark of *Cassia occidentalis*) was collected from Jinnah Garden Lahore. Herbarium specimen was prepared and placed in Herbarium of Lahore College for Women University, Lahore, Pakistan. Bark was air dried, finely powdered and stored in air tight jars. Microwave assisted extraction in present study was carried out on Haier microwave (HGN-45100EB). Parameters studied were power level (300, 500, 700 and 900W), time (10, 20, 30, ..., 100 seconds), solvent type (Ethanol, Water and n-hexane) and solvent quantity (5-15ml). Each time 1g plant material was taken. For soxhlet extraction parameters optimized were time of extraction (5, 10, 15 and 20 hours), Solvent type (Ethanol, Water, n-hexane) and quantity of solvent (150, 200, 250 and 300 mL). Each time 30 grams of plant sample was taken. After that percentage of extract was determined and each extract was quantified for phenolics, flavonoids and alkaloids.

Estimation of Phenolics

Spectrophotometric assay was used to estimate phenolics in the extract [14]. Each time 1 ml of sample (1mg/1ml) + 1 ml of

Folinand Ciocalteu's phenol reagent was mixed and left to stay for 3 minutes, followed by the addition of 1 ml of saturated Na_2CO_3 and total volume was raised up to 10ml by distilled water. Reaction mixture was kept in dark for 1.5 hours and then

absorbance was checked at 725 nm. Standard curve of Gallic acid (Fig. 1) was constructed by using dilutions of Gallic acid. Phenolics were represented as μg eq of Gallic acid.

Figure 1. Standard absorption graph of Gallic acid

Estimation of flavonoids

Flavonoids present in the extracts were also determined by Spectrophotometric method [15]. In this method, 1 ml of sample (1mg/ml) was mixed with 5 ml of distilled water and 0.3 ml of 5% sodium nitrite and 0.6 ml of aluminum chloride was added and reaction

mixture was allowed to stand for 6 minutes. After that 1.5 ml of distilled water and 2ml of 1M NaOH were added and after thorough mixing, absorbance was checked at 510nm, where Catechin was used as a standard (Fig. 2).

Figure 2. Standard absorption graph of Catechin

Estimation of Alkaloids

Spectrophotometric method was also used to study the alkaloid content of extract [16]. For this purpose, 0.5g of extract + 1ml of ethanol+ 2ml of Dragon Droff reagent were mixed well. Precipitates formed were dissolved in 0.5ml of disodium sulphide with 2-3 drops of conc. Nitric acid. Brown ppts formed were mixed in 2ml of Thiourea and absorbance was checked at 435 nm.

Dragondroff's reagent was used to calculate the standard curve and the results were expressed as mL of Dragondroff's equivalents (DEs) per mg of extract (Fig 3). The data produced from all above experiments was analyzed by applying ANOVA and Duncan's multiple range test at 5% level of significance using COSTAT computer software.

Figure 3. Standard absorption graph of Dragondroff reagent

In present research work, microwave assisted extraction and Soxhlet extraction of *Cassia occidentalis* L (bark powder) was done followed by estimation of phenolics, flavonoids and alkaloids. There was no significant effect of timing on MAE at power level 300 W. The highest amount of extraction was 140, 190 and 170 mg per gram of plant material at power levels 500W, 700 W and 900 W respectively; and timings optimized were 70, 70 and 30 seconds, respectively. Results prominently showed that on increasing the power level from 500-700 W, amount of extract started to increase (Table 1); while a further increase in the power level, extract started to decrease. In the

present research work, effect of solvent nature on extraction was also studied and results showed that ethanol has greater capability of extraction from *Cassia occidentalis*' bark powder as compared to water and hexane, at all power levels i.e., 300, 500 and 900 W, maximum extract was obtained from ethanol (Fig 4).

While 10 mL/g of solvent was optimized quantity for optimum microwave assisted extraction of *Cassia occidentalis*.

Jaitaket *et al.*, 2009 and Javad *et al.*, 2014 obtained higher amounts of stevioside from microwave assisted extraction of *Stevia rebaudiana* in a lower power level and higher timing combination [17, 18]. Higher amounts

of curcumin have also been reported by Mandal *et al.*, 2008 from microwave assisted extraction of *Curcuma longa* L. They were also in the favor of low power and higher time of microwaves irradiation [19]. Almost similar results were presented by Shu *et al.*, 2003 by microwave assisted extraction of ginseng roots [20]. Microwave assisted extraction is mainly affected by two factors i.e., power level of microwaves and time of irradiation. These two factors have proven

effect on the final product of microwave assisted extraction of bioactive components. High time to low power combination is an often used protocol for optimizing extraction of any compound using microwave assisted extraction because higher energy levels increase kinetic energies up to the level where extract may be burnt and similarly longer exposures to microwaves can also damage the molecules [21].

Table 1. Optimization of time and power level of microwave assisted extraction from bark of *Cassia occidentalis*

Sr. #	Time of Extraction (sec)	Extraction from bark of <i>Cassia occidentalis</i> in microwaves			
		300 W	500 W	700 W	900W
1	10	70 ^{cd} ±4.00	70 ^d ±7.00	70 ^h ±4.00	130 ^b ±2.0
2	20	80 ^c ±5.00	90 ^c ±1.00	130 ^e ±1.00	130 ^b ±2.0
3	30	88 ^c ±1.0	110 ^b ±3.00	140 ^d ±3.00	170 ^a ±3.0
4	40	100 ^{ab} ±1.0	100 ^b ±2.00	150 ^c ±5.00	130 ^b ±3.0
5	50	100 ^{ab} ±3.0	100 ^b ±4.00	140 ^d ±2.00	120 ^c ±1.0
6	60	102 ^{ab} ±1.0	100 ^b ±1.00	160 ^b ±2.00	100 ^d ±1.0
7	70	102 ^{ab} ±0.09	140 ^a ±1.00	190 ^a ±2.00	100 ^d ±4.0
8	80	110 ^a ±1.0	80 ^c ±9.00	110 ^e ±3.00	80 ^e ±3.0
9	90	110 ^a ±1.00	80 ^c ±1.00	130 ^e ±1.00	60 ^e ±4.0
10	100	110 ^a ±4.00	60 ^d ±3.00	120 ^f ±1.00	60 ^e ±1.0

Each value is mean of five replicate with standard error (mean ± S. E). Means within a column not sharing a common superscript differ significantly (P<0.05) according to Duncan's new multiple range test

Figure 4: Effect of solvent nature on microwave assisted extraction of *Cassia occidentalis* L.

Present study also showed that soxhlet extraction produced only 160mg of extract per 30 gram or 5.33mg per gram of plant material after 15 hours of extraction from ethanol (Table 2). Figure 5 shows the efficiency of ethanol for soxhlet extraction of *Cassia occidentalis* L. Soxhlet extractions usually take longer times to accomplish; this may be due to static condition of plant material which means there is no agitation. Agitation basically provides more exposure of the matrix and extracting solvent which in

turn leads towards the breakage of more bonds and release of more products in the environment. Narrow position of plant material in the thimble of soxhlet extractor is another reason which decreases the chances of complete contact of the solvent and the plant sample. Due to longer time of extraction there is increased evaporation of solvent, so consumption of solvent is increased along with the heating energy. Longer heating times also destroy chemical components of the extract [22].

Table 2. Optimization of time of soxhlet extraction from bark of *Cassia occidentalis*

Sr. #	Time of extraction Hours	Amount of extract	
		mg/30g	mg/g
1	5	120 ^c ±3.37	4.00 ^c ±1.12
2	10	131 ^b ±8.43	4.33 ^b ±2.47
3	15	160 ^a ±4.51	5.33 ^a ±1.50
4	20	110 ^d ±3.81	3.33 ^d ±1.27

Each value is mean of five replicate with standard error (mean ± S. E). Means within a column not sharing a common superscript differ significantly (P<0.05) according to Duncan's new multiple range test

Figure 5. Effect of solvent nature on soxhlet extraction of Bark of *Cassia occidentalis* L.

Soxhlet extractions usually take longer times to accomplish; this may be due to static condition of plant material which means there is no agitation. Agitation basically provides more exposure of the matrix and extracting solvent which in turn leads towards the breakage of more bonds and release of more products in the environment. Narrow position of plant material in the thimble of soxhlet extractor is another reason which decreases the chances of complete contact of the solvent and the plant sample. Due to longer time of extraction there is increased evaporation of solvent, so consumption of solvent is increased along with the heating energy. Longer heating

times also destroy chemical components of the extract [22].

Present study also revealed higher amounts of phenolics (170 µg eq. of Gallic acid), flavonoids (177 µg eq. of Catechin) and alkaloids (3.1 µg equivalent of Dragondroff) in microwave assisted extraction (Table 3) as compared to soxhlet extraction (97 µg eq. of Gallic acid, 118 µg eq. of Catechin and 1.6 µg equivalent of Dragondroff), respectively (Table 4). When amount of these phytochemical was compared, it was found that amount of phenolics, flavonoids and alkaloids were higher in microwave assisted extraction as compared to extracts of soxhlet extraction (Table 4).

Table 3. Estimation of phytochemical in microwave assisted extracts of *Cassia occidentalis*

Sr #	Power level (W)	Time (sec)	Phenolics (µg equivalent of Gallic acid)	Flavonoids (µg equivalent of Catechin)	Alkaloids (mg equivalent of Dragondroff reagent)
1	500	10	51.65 ^h ±1.00	48.0±2.11	0.6 ^{fg} ±0.03
		30	20.37 ^j ±2.33	120 ^e ±0.39	0.4 ^g ±0.09
		50	102.62 ^g ±9.02	141 ^e ±2.47	0.7 ^f ±0.001
		70	67.75 ^h ±4.31	88 ^g ±1.98	0.35 ^g ±0.01
		90	55.75 ^h ±1.04	48 ^h ±4.09	0.25 ^g ±0.05
		100	49.00 ⁱ ±3.34	93 ^g ±3.33	0.25 ^g ±0.05
2	700	10	116.12 ^f ±1.76	103 ^f ±3.09	1.1 ^e ±0.05
		30	141.37 ^c ±1.39	140 ^d ±8.09	1.25 ^e ±0.01
		50	137.62 ^d ±5.34	171 ^b ±2.53	1.7 ^d ±0.03
		70	139.12 ^{cd} ±4.69	160 ^c ±8.65	2.2 ^c ±0.09
		90	125.00 ^e ±3.28	110 ^f ±2.74	1.55 ^e ±0.01
		100	118.18 ^f ±6.66	105 ^f ±1.09	0.8 ^f ±0.02
3	900	10	150.62 ^b ±4.33	49 ^h ±2.71	2.1 ^c ±0.02
		30	170.87 ^a ±6.10	100 ^f ±8.55	2.2 ^c ±0.08
		50	148.75 ^b ±7.12	148 ^d ±3.09	2.45 ^b ±0.06
		70	141.95 ^c ±2.75	177 ^a ±1.44	3.2 ^a ±0.01
		90	136.5 ^d ±4.11	138 ^e ±9.12	2.9 ^{ab} ±0.01
		100	124.09 ^e ±1.15	121 ^e ±3.29	0.6 ^{fg} ±0.05

Each value is mean of five replicate with standard error (mean ± S. E). Means within a column not sharing a common superscript differ significantly (P<0.05) according to Duncan's new multiple range test

Table 4. Estimation of phytochemical in soxhlet extracts of *Cassia occidentalis* L.

Sr. No.	Time of extraction (Hours)	Phenolics (μg equivalent of Gallic acid)	Flavonoids (μg equivalent of Catechin)	Alkaloids (mg equivalent of Dragondroff reagent)
1	5	55.12 ^d ±2.56	102 ^c ±4.44	0.6 ^d ±0.04
2	10	91.25 ^b ±2.90	120 ^a ±3.92	1.45 ^b ±0.01
3	15	96.75 ^a ±0.09	110 ^b ±8.20	1.55 ^a ±0.09
4	20	84.75 ^c ±3.33	80 ^d ±4.32	1.1 ^c ±0.013

Each value is mean of five replicate with standard error (mean $\pm$ S. E). Means within a column not sharing a common superscript differ significantly ($P < 0.05$) according to Duncan's new multiple range test

Phenolics are the key bioactive compounds isolated from medicinal plants. Plant polyphenols have prominent antimutagenic activity due to their antioxidant potential [23]. Rafiee *et al.*, 2011 used microwave assisted extraction to isolate the phenolics from leaves of Olive and also made a comparison with maceration. They found the result at end that in all respects and for all parameters, microwave assisted extraction was the better choice for extraction of phenolics [24]. The extraction from microwave assisted extraction was of low cost and had high quality extraction rate. Victorio *et al.*, 2009 separated flavonoids from leaves of *Alpini azerumbet* Pers. by using different extraction techniques such as maceration, soxhlet, microwave assisted extraction and ultrasonic extraction. They used ethanol and distill water were used for detection of flavonoids. Results showed rapid and maximum yield of flavonoids as compared to other methods of extraction [25]. Moreover ethanol is a polar solvent. As microwave assisted extraction depends on the dielectric susceptibility of solvent and matrix, better recoveries can be there by using polar solvents with high dielectric constant. But very dry samples as well as very wet samples with non-polar solvents like n-hexane decrease the efficiency of

microwave assisted extraction. Non-polar solvents usually do not respond to the microwaves due to their lower dielectric constant and dissipation factor in comparison to the polar solvents, so no heat is transferred and no or a very little extraction goes on there. Water has a higher dielectric constant but it is sometimes so rapidly heated in microwaves that it destroys the chemical structure of compounds. No doubt there is a clear relationship between the chemical structure and the stability of phenolic compounds in solvents, so it also affects the efficacy of microwave assisted extraction depending on the plant source used. Microwave-assisted extraction may also be comparable to other modern extraction techniques such as supercritical fluid extraction due to its process simplicity and low cost [26, 27].

Conclusion

It was concluded from present study that Microwave assisted extraction is a rapid, efficient, cost effective and reliable method of extraction for the extraction of phenolics, alkaloids and flavonoids from *Cassia occidentalis*. This method can be feasibly suggested to local herbal industry for cost effective and efficient extraction of phytoconstituents of *Cassia occidentalis*.

Authors' contributions

Conceived and designed the experiments: S Javad. Performed the experiments: S Younis. Analyzed the data: S Shaheen. Contributed reagents/materials/analyses tools: F Khan. Wrote the paper: S Javad.

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