International Journal of Pharma and Bio Sciences V1(1)2010

MICROWAVE ASSISTED EXTRACTION OF *ARTEMISIA PALLENS* FOR TYROSINASE INHIBITORY ACTIVITY

KANIMOZHI. P[*1], *K. ILANGO*[2], *ANUPAM CHOWDARY*[1], *ARUN. B*[1], *AMMAR MOHAMMED MUSA ADAM*[1] *and A. NETHAJI*[1].

[1]Department of Pharmacognosy, SRM College of Pharmacy, SRM University, Kattankulathur - 603203.

[2]Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM University, Kattankulathur – 603203.

**** Corresponding author*** kanimozhiswami@gmail.com

ABSTRACT

The present study deals with the application of microwave as an extraction tool for extraction of phytoconstituents present in *Artemisia pallens* wall (compositae) and determination of tyrosinase inhibitory activity which serves as an useful target in the treatment of hyper pigmentation skin disorder. The percentage yield of extract obtained by microwave assisted extraction (MAE) of plant was found to be highly significant when compared to soxhlet extraction (SE) method. The tyrosinase inhibitory activity of MAE was found to be highly significant ($p < 0.0001$) when compared with that obtained by SE method.

KEY WORDS

Microwave assisted extraction, *Artemisia pallens*, Tyrosinase inhibition and Hyper pigmentation.

INTRODUCTION

Medicinal plants provide a wide array of hope through its phyto compounds which are behind to act in synergistic manner, providing excellent healing touch with no desirable side effects, provided its quality is assured. The extraction and characterization of active phyto compounds give rise to some increased activity profile drugs[1]. It forms the first basic step in medicinal plant research as it is a starting point for isolation and purification of therapeutically active constituents present in plant.

MICROWAVE ASSISTED EXTRACTION OF *ARTEMISIA PALLENS* FOR TYROSINASE INHIBITORY ACTIVITY

Recent times have witnessed the use and growth of new techniques with shortened extraction time and decreased solvent consumption, increased pollution prevention and special care for thermo labile substances[2]. Microwave assisted extraction involves non ionizing electromagnetic waves of frequency between 300MHZ to 300 GHZ which are generated from unit made up of two oscillating perpendicular fields namely electric and magnetic fields which are responsible for heating[3]. This involves two phenomenon namely ionic conduction and dipole rotation. The plant under investigation in the current study, *Artemisia pallens* wall (compositae) is an aromatic perennial shrub, hairy, pubescent erect, stem, angled, ribbed. Traditionally used in the treatment of diabetes[4], inflammation[5] and urinary problem[6]. The plant was found to exhibit antibacterial, antifungal[7], antiviral[8], anticoagulant and antihyperlipidemic activities[9]. Hence in the current study an attempt was made to study the tyrosinase inhibitory potential of *Artemisia pallens*. As tyrosinase enzyme (copper containing polyphenol oxidase) is responsible for pigmentation of skin, eyes and hair[10] through enhancement of melanin production, the determination of tyrosinase inhibitory activity would be useful in the treatment of skin pigmentation disorders.

MATERIALS AND METHODS

The plant material was collected from Chennai and authenticated at National Institute of Herbal Science PARC by botanist Prof. P. Jayaraman (Reg. No. PARC/2009/286). The collected material was air dried and then powdered.

(i) Extraction

Soxhlet method of extraction

10gms of powdered plant material was exhaustively extracted with 200ml ethanol by continuous hot percolation method. After three hours the extract was concentrated to 3/4th of volume to get a concentrate.

Microwave assisted method of extraction

Extraction was carried out in domestic microwave oven (Kenstar Frequency – 40GHZ, Power grill – 1300). About 10gms of dried powdered plant material was mixed with 200ml of ethanol and was radiated in microwave oven at varying time intervals such as 30, 60, 90, 120, 150 seconds respectively (30 sec radiation and 1min off) to keep temperature not rise above 60^0 C. The infusions were allowed to cool down to room temperature, filtered and stored in refrigerator at 4^{0}C for the determination of total phenolics [11].

(ii) Determination of total phenolics

Folin-ciocalteau method was used to determine total phenolics. About 10mg of sample was made upto 10ml using methanol: water in the ratio of 20: 30. 1ml of the above solution was treated

MICROWAVE ASSISTED EXTRACTION OF *ARTEMISIA PALLENS* FOR TYROSINASE INHIBITORY ACTIVITY

with 5ml of folin's ciocalteau phenol reagent (1:2) and 4ml of 7.5% sodium carbonate solution were added and mixed well. Standard solution of gallic acid in methanol (10 to 100µg/ml) were prepared and treated in identical manner. After 1hour 30 minutes, the absorbance of test and standard solutions at 750nm was measured on Lambda25 (Perkin Elmer UV Spectrophotometer). The total phenolic content was calculated graphically using Gallic acid standard curve and the results were expressed as gallic acid equivalent [12].

(iii)Determination of reducing power

About 2.5ml of extract was treated with 2.5ml of phosphate buffer (0.2m, pH 6.6), 2.5ml of 1% potassium ferricyanide (10mg/ml) and was then incubated at 50°C for 20 minutes. It was then rapidly cooled and treated with 2.5ml of 10% trichloroacetic acid and then centrifuged at 6500 rpm for 10minutes. The supernatant was then treated with distilled water and 0.5ml of ferric chloride solution. It was then allowed to stand for 10minutes. The absorbance was measured at 700nm using L- ascorbic acid as standard [13].

(iv)Determination of tyrosinase inhibitory activity

About 20µL of mushroom Tyrosinase solution (1000U/ml) was treated with 0.1M phosphate buffer (pH 6.8) and 100µL of test sample solution containing 20µL of plant extracts. Then sample solution without enzyme was also prepared in the same manner. Blank solution with and without enzyme was also prepared. About 20µL of 0.55mM L-DOPA solutions as substrate was added into every sample and blank. These assay mixtures were incubated at 37 °C for 10minutes, the amount of dopachrome produced in the reaction mixture was measured at 475nm (Perkin Elmer UV Spectrophotometer). The percentage inhibition of Tyrosinase inhibitory activity was calculated using following formula:

Percentage Tyrosinase inhibition

$$= \frac{(A - B) - (C - D) \times 100}{(A - B)}$$

Where A = Absorbance of blank solution with enzyme; B = Absorbance of blank solution without enzyme; C = Absorbance of sample solution with enzyme; D = Absorbance of sample solution without enzyme [14, 15].

Statistical analysis

The percentage inhibitory activity of the extracts obtained by two methods was analyzed statistically by one way ANOVA followed by Dunnets test at $P < 0.0001$.

RESULTS

(1) Percentage yield of extracts obtained by different extraction methods

MICROWAVE ASSISTED EXTRACTION OF *ARTEMISIA PALLENS* FOR TYROSINASE INHIBITORY ACTIVITY

The results of percentage yield of extracts obtained by different methods have been shown in table 1. The maximum percentage yield was obtained by microwave assisted extraction (MAE) within 90 seconds which was high when compared with that obtained by soxhlet method of extraction obtained in 3 hours.

Table 1

Percentage yield of extracts

Extraction	Percentage yield
Microwave assisted extraction (MAE)	
30 seconds	4.7%
60 seconds	6.0%
90 seconds	6.7%
120 seconds	3.9%
150 seconds	2.6%
Soxhlet extraction (SE)	
3 hours	6.2%

(2) Total phenolic content

The extract obtained by MAE was found to contain maximum amount of phenolic content when compared with that obtained by SE method as shown in table 2.

Table 2

Total phenolic content

Sample	Total phenolics (µg/mg)
M1	30
M2	40
M3	109
M4	20
M5	5
S	30

M, the extracts obtained by means of microwave assisted extraction at different time duration mentioned as M1 = 30secs, M2 = 60 secs, M3= 90 secs, M4= 120 secs, M5= 150 secs and S, extract obtained by means of Soxhlet extraction = 3 hours.

(3) Reducing power

Similarly the reducing power indicated by an increase in absorbance value was evaluated for both the extracts and MAE extract was found to posess more reducing ability as shown in table 3.

MICROWAVE ASSISTED EXTRACTION OF *ARTEMISIA PALLENS* FOR TYROSINASE INHIBITORY ACTIVITY

Table 3

Reducing power of extracts obtained by different extraction methods

Concentration	Standard Ascorbic acid	MAE	SE
30	0.1215±0.0091	0.0028±0.0004	0.0027±0.0003
45	0.1777±0.0030	0.0741±0.0034	0.0242±0.0468
60	0.2451±0.0030	0.1662±0.086	0.0933±0.047

The reducing power of extract prepared by microwave assisted extraction method was found to be highly significant at $p < 0.0001$ when compared with that obtained by soxhlet method. MAE – Extract obtained by Microwave assisted extraction; SE – Extract obtained by Soxhlet extraction.

(4) Tyrosinase inhibitory activity

The Tyrosinase inhibitory activity of MAE extract was found to be more than that of conventional SE method. The results had been shown in table 4.

Table 4

Tyrosinase inhibitory activity

Extraction method	Percentage inhibition
MAE	75.49± 0.32%
SE	65.85±0.13%

The percentage tyrosinase inhibitory activity of extract prepared by microwave assisted extraction method was found to be highly significant at p< 0.0001 when compared with that obtained by soxhlet method. MAE – Extract obtained by Microwave assisted extraction; SE – Extract obtained by Soxhlet extraction.

DISCUSSION

MAE was considered to be the best method of extraction than SE method due to significant reduction in extraction time which varied between few seconds to few minutes, improved extraction yield and suitability for thermo labile constituents. Also the results obtained showed the extract of *Artemisia pallens* obtained by MAE to possess increased reducing power which forms the basis for exploring the Tyrosinase inhibitory potential of plant. Tyrosinase is a copper containing enzyme hence any substance which reduces this metal ion was considered as an effective Tyrosinase inhibitor. The reducing power reported might be due to phytoconstituents such as phenolics, flavonoids and also other constituents which

MICROWAVE ASSISTED EXTRACTION OF *ARTEMISIA PALLENS* FOR TYROSINASE INHIBITORY ACTIVITY

are present in plant. The possible mechanism underlying behind the Tyrosinase inhibitory ability might be chelation of copper ion present in Tyrosinase enzyme by phytoconstituents and thereby suppression of tautomerisation to dopochrome by the plant extract, thereby the plant extract acts as reducing agents on melanin intermediates by blocking oxidation chain reaction at various points from Tyrosinase/ DOPA to melanin and hence causing reduction of skin pigmentation.

CONCLUSION

Artemisia pallens was found to be rich in phenolic compounds consisting of hydrophobic part which would have acted as competitive inhibitor on the enzyme tyrosinase and thereby on melanin synthesis. Hence the determination of tyrosinase inhibitory potential of *Artemisia pallens* paves a way for development of skin whitening agents. The investigation of plant extract on human melanocytes has to be performed to confirm the anti hyper pigmentation effect of the plant under investigation.

ACKNOWLEDGEMENTS

Authors are grateful to Dr.R.Shivakumar, Pro-Vicechancellor, S.R.M.University and Dr.K.S.Lakshmi, Dean, College of Pharmacy, S.R.M.University, for providing necessary facilities to carry out this work.

REFERENCES

1. Huie. C.W, A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. Analytical and Bio analytical Chemistry, 373(1): 23 – 30, (2002).
2. Letellier. M, Budzinski. H, Optimization by factorial design of focused microwave assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. Journal of Analytical Chemistry, 364(3): 228 – 237, (1999).
3. Letellier. M, Budzinski. H, Microwave assisted extraction of organic compounds. Anaiusis, 27(3): 259 – 271, (1999).
4. Swanston Flatt. S.K, Flatt. P.R, Day C. Bailey, Traditional dietary adjuncts for the treatment of diabetes mellitus. Proceedings of nutritional society, 50(3): 641 – 651, (1991).
5. Mascolo. N, Autore. G, Caposso. F, Menghini. A, Fascula. M.P, Biological screening of Italian medicinal plants for anti-inflammatory activity. Phytotherapy research, 1(1): 28-31, (1987).
6. Bolek. B.R, Ethnobotany of costanoan Indians, based on collections by John P Marrington. Economic Botany, 38(2): 240 - 255, (1984).
7. Mehrota. S, Rawat A.K.S, Shome, Antimicrobial activity of the essential oil of some Indian

MICROWAVE ASSISTED EXTRACTION OF *ARTEMISIA PALLENS* FOR TYROSINASE INHIBITORY ACTIVITY

Artemisia species. Fitotherapia, 64(1): 65 – 68, (1993).

8. Natake M, Kanayawa K, Miyuno. M, Veno.N, Kobayashi. T, Minamotao. S, Herb water extracts markedly suppress mutagenecity of Trp – P-2. Agricultural and biological chemistry, 53(5):1423-1425, (1989).

9. Yazdean P, Saee. A, Effects of aqueous tarragon, *Artemisia dracunculus* extract on lipid and coagulatory parameters in rats. Biomedical letters, 59(233): 137 – 141, (1999)

10. Kral J.K, Hopkins. T.L, Tyrosinase inhibitors for insect cuticle tanning. Archives of insect Biochemistry and Physiology, 6(4): 279 – 301, (1987).

11. Pham Thanh Quan, Tong Van Hang, Microwave assisted extraction of polyphenols from fresh tea shoot. TAP CHI TRIEM KH & CN, 9(8): 69 - 75, (2006).

12. Shahidi. F, Wanasundara.P.K.J.P.D, Phenolic antioxidants. Critical reviews in food science and nutrition. 32(1): 67 – 103, (1992).

13. Spanos. G.A, Wrolstad. R.E and Heatherbell. D.A, Influence of processing and storage on the phenolic composition of apple juice. Journal of Agriculture and food chemistry, 38(7): 1572 – 1579, (1990).

14. Pio. X.L, Baek. S.H, Tyrosinase inhibitory furanocoumarin from *Angelica dahurica*. Biological and Pharmaceutical Bulletin, 27(7): 1144 – 1146, (2004).

15. Pomerantz. S.H. Purification and properties of tyrosinase isoenzymes from hamster melanoma, Yale Journal of Biology and medicine, 46(5): 541-552, (1973).