International Journal of Advance Research and Innovation
Vol. 10(1), Jan-Mar 2022, pp. 33-37
Doi: 10.51976/ijari.1012206

Article Info

Received: 25 Jan 2022 | Revised Submission: 27 Feb 2022 | Accepted: 02 Mar 2022 | Available Online: 15 Mar 2022

Extraction and Identification of Active Components from Herbs for their Application for Prevention of Poly Cystic Ovary Syndrome (PCOS)

Virpal Kaur, Parul Thapar** and Saruch****

ABSTRACT

Polycystic ovary syndrome (PCOS) is a common gynecological endocrine disorder that causes reproductive and metabolic disturbances. PCOS is more common in females who have obesity and it can also be a genetic disorder in some females. During a study on teen age 17-19 years aged girls in Thailand, the prevalence rate of PCOS syndrome was 5.29 %. While, the studies in Iran had shown the prevalence rate to be 8.3% to 11.4% (Naz et al, 2019). PCOS can be treated by allopathic medicines but they have number of side effects in women like acne and menstrual irregularity. Therefore, in this study an alternative solution has been taken to prevent PCOS by the application of herbs. In this study, 7 samples of herbs were considered. Sample of Cinnamon (dalchini) was collected from a field of Punjab Agriculture University Ludhiana; Curcuma longa (turmeric), Mentha piperita (pippermint) and Allium sativum (garlic) were taken from Gurudwara Bhora Sahib (Jagraon); Ocimum sanctum (tulsi), Azadirachta indica (neem) and Casearia esculanta (saptrangi) were collected from Farm house of CT university campus (Mullanpur). These samples were dried for 48 hours at temperature 38˚C. The process of solvent extraction was used to extract the active components. To identify the extracted constituents from herbs, thin layer chromatography (TLC) was done. Some biochemical tests were performed, to confirm the presence of the identified components. From these 7 samples the active components that were successfully identified were cinnamaldehyde, allicin, estradiol and diterpenoids, alkaloids and flavonoids. These components will be further used for the preparation of herbal medicine against.

Keywords: *Polycystic ovary syndrome; Estrogen hormone; Obesity; Insulin resistance; Herbs and solvent extraction.*

1.0 Introduction

Polycystic ovary syndrome (PCOS) is a common gynecological endocrine disorder that causes reproductive and metabolic disturbances. PCOS is more common in females who have obesity and it can also be a genetic disorder in some females (Hadi et al, 2020). The diverse nature of PCOS was evident even from the first description of the syndrome by Stein and Leventhal who in their original report described seven women with different problems like obesity, hirsutism, acne and amenorrhea associated with enlarged bilateral polycystic ovaries (Conway et al, 2014). PCOS has no particular cure but changes in lifestyle by pharmacological therapies are helpful as cure (Hadi et al, 2020). When PCOS prevalence rate based on the National Institute of Health (NIH) criteria was conducted in Australia on 16 to 29 years old Australian youth, it was found to be 12% (Ybarra et al, 2018). According to Rotterdam criteria, the effect of PCOS on Chinese females was 5.6% which was considered as the minimum prevalence rate whereas on Iranian women, the effect was 19.5% (Ding et al, 2017). A study was conducted in Tamil Nadu with adolescent women that found the effect to be near about 18 percent for PCOS.

The study also mentioned that females of urban area were more affected with PCOS as compared to rural females. A similar study was conducted in Mumbai. It was found that the prevalence of PCOS

**Correrspondign author; CT University, Ludhiana, Punjab, India (Email: dhaliwalg185@gmail.com)*
***Department of Food Technology, Gandhi Institute of Technology and Management (GITAM) Deemed University, Hyderabad, Telangana, India (E-mail: parul.thapar@yahoo.com)*
****CT Institute of Pharmaceutical Sciences, Shahpur Campus, Jalandhar, Punjab, India (Email: suruchinitj15@gmail.com)*

was 22.5% in the urban community by Rotterdam criteria and 10.7% by the Androgen Excess Society criteria (Ganie et al, 2019). The condition of PCOS can be treated by allopathic medicines but they have number of side effects in women like weight gain, acne, menstrual irregularity, metabolic changes and unhealthy pregnancy (Naz et al, 2019). Plants have become a source of medicinal agents for thousands of years (Sanguri et al, 2012; Paul and Sainsburg, 1991). Many plant species that are endowed with phytochemicals and strong antimicrobial properties have been documented pharmacologically and therapeutically throughout the world (Thapar and Garcha, 2017; Bashir et al, 1992). In the present study, an alternative solution has been taken to prevent PCOS by the.

2.0 Objective of the Study

Extraction and identification of active components from different plants as herbs.

3.0 Materials and Methods

3.1 Sample collection

Seven different samples of herbs were collected for the study. Cinnamon (dalchini) was collected in the form of plant at the outside of PAU Ludhiana; Curcuma longa (turmeric), Mentha piperita (pippermint) and Allium sativum (garlic) were taken from Gurudwara Bhora Sahib (Jagraon); Ocimum sanctum (tulsi) and Azadirachta indica (neem) were collected from Farm house (Jagraon) and Casearia esculanta was taken from CT university campus (Mullanpur). These fresh plant samples were washed with tap water and then distilled water. Then they were dried at 38˚C for 48 hours in hot air oven.

3.2 Conversion into powder form

All the seven samples were separately meshed into fine powder with pestle and mortar. The powdered material was kept in seven air tight jars in refrigerator at 4˚C for 24 hours.

3.3 Extraction of active components by solvent method

After 24 hours, 5 grams of the samples of seven fine powders were kept in formaldehyde: Alcohol: Diethyl ether solution at the concentration of 60:20:40 respectively. The mixture was kept undisturbed at room temperature for 24 hours in sterilized flask and it was covered with aluminium foil to prevent the evaporation of the solvent. After 24 hours, the samples were filtered using filter paper of pore size 0.22 µm and the filtrates containing active components were stored till further use.

3.4 Identification of compounds by thin layer chromatography (TLC)

Thin layer chromatography (TLC) is the simplest and most economic chromatographic method for detection and separation of small quantities of organic and inorganic compounds (Misra and Pachauree, 2002). Chromatography involves separation of compounds from a mixture, based on the difference in their partition coefficient between mobile phase and stationary phase (Aggrawal and Behri, 2015). For TLC, the plate was prepared by adding silica gel. A thin mark was made at the bottom of the TLC plate with a pencil. Seven spots were marked at equal distances. All the seven filtrates were then applied on these spots. The TLC chamber was filled with mixture of suitable solvents like diethyl ether, ethyl acetate, chloroform, toluene, hexane and methanol. The prepared plate was put inside the TLC chamber. Then closed the chamber and allowed the solvent to travel across the plate for 2 hours. After 2 hours, the plate was taken out and allowed to dry. The movement of the active components from all the 7 samples on the plate was analyzed under U.V.

3.5 Confirmatory tests for the identified components

For confirmation of the identified components from obtained RF value, some biochemical tests were performed as:

- FeCl3 test for Alkaloids: In 2 ml of the each test sample, one drop of FeCl3 solution was added to test for the formation of yellow precipitates (Marka et al, 2013).
- Alkaline reagent test for Flavonoids: In 2 ml of the each test sample, 2 drops of sodium hydroxide solution was added to test for the formation of intense yellow colour. Then, added to test for the change into colourless solution.
- Cinnamaldehyde test: In 2 ml of the each test sample, few drops of alcohol and ferric chloride was added to test for the formation of pale green or brown colour.

- Concentrated Sulphuric acid test for glycoside: In 2 ml of the each test sample, 2 drops of conc.H2SO4 was added to test for the appearance of reddish colour.

4.0 Results and Discussion

4.1 Solvent extraction

From all the seven samples, seven different filtrates containing active components were extracted. The seven filtrates had different colour and odour. These are shown in the Table 1 and Figure 1.

In a study, phytochemical screening of leaf, stem, root and seed extracts of Arachis hypogaea was done using different solvents. Fluorescence analysis of all the extracts was carried out under UV and normal light for identification of phytochemicals present in different coloured extracts. It was found that the leaf extract of the plant separated from benzene showed light green colour at normal and brown colour under U.V. for the presence of alkaloids (Marka et al, 2013).

Figure 1: Herbal Filtrate

Table 1: Colours of the Extracted Filtrate

S. No.	Herbs filtrate after solvent extraction	Colours of the extracted filtrate
1.	Cinnamon	Cream
2.	Curcuma longa	Orange
3.	Mentha piperta	Brown
4.	Allium sativum	Brown
5.	Ocimum sanctum	Brown
6.	Azadirachta indica	Light Brown
7.	Caseria esculanta	Brown

4.2 Identification of active components through thin layer chromatography (TLC)

During TLC, the distance travelled by the solvent and distance travelled by solute for all the seven samples was recorded. The RF (retention factor) value was estimated by using the formula Rf = distance travelled by solute/ distance travelled by solvent. According to the Rf value observed for the components present in the 7 samples, the comparison of these values for the identification of the active components was done using RF value chart (Bhatia et al, 2015). These values are mentioned in the Table 2. This test was conducted in three trials. The statistical analysis was done using ANOVA- single factor. The P- value of ≤ 0.05 was considered appropriate for the test. These are shown in the Table 3.

4.3 Biochemical tests for the identification and confirmation of the components

After comparison with the RF value chart, it was found that the obtained values for some extracts were less or more than that of the theoretical values.

Table 2: RF Values of the Extracted Filtrate

S. No.	Herbs	Solvents used in TLC chamber	Obtained average RF value	Theoretical Rf value in different solvents
1.	Cinnamon	Chloroform, ethyl acetate and formic acid (5:4:1)	0.35	0.86- beta carotene, 0.31- chloroform, methanol & ethanol, 0.35- CCl4, 0.32- ethyl acetate
2.	Curcuma longa	Diethyl ether	0.11	0.3- petroleum ether
3.	Mentha	Hexane	0.11	0.13- acetone, 0.13- acetone, 0.14- ethylacetate
4.	Allium sativum	Chloroform and methanol (99:1)	0.14	0.84-petroleum ether, 0.83- chloroform, 0.81- ethanol, 0.82- ethyl acetate,0.21- methanol
5.	Ocimum sanctum	Toluene and Ethyl acetate (93:7)	0.05	0.84-petroleum ether, 0.83- chloroform, 0.81- ethanol, 0.82- ethyl acetate
6.	Azadirachta indica	Chloroform, methanol and ethanol (5:4:1)	0.04	0.41- methanol, 0.35- CCl4, 0.34- acetone, 0.31- chloroform, methanol & ethanol 0.32- ethyl acetate
7.	Caseria esculanta	Ethyl acetate	0.3	0.13-acetone, 0.14- ethylacetate

So, to confirm the identification of the components in the extract, some biochemical tests were performed. The results are shown in the Table 4. Out of the seven extracts, the confirmed identified compounds obtained were cinnamaldehyde, allicin, estradiol and diterpenoids, alkaloids and flavonoids. A study by Marka et al, 2013, confirmed the leaf extract of the plant confirmed the presence of alkaloids through FeCl3 test.

Table 3: Statistical Analysis using ANOVA - Single Factor

Groups	Count	Sum	Average	P-value
Cinnamon	3	1.07	0.35	0.0043
Curcuma longa	3	0.33	0.11	
Mentha	3	0.326	0.11	
Allium sativum	3	0.415	0.14	
Ocimum sanctum	3	0.166	0.05	
Azadirachta indica	3	0.108	0.04	
Caseria esculanta	3	0.86	0.3	

Table 4: Confirmatory Tests for the Identified Components

S. No.	Herbs	Test	Obser-vation	Result	Confirmed identified components
1.	Cinnamon	Cinnamic aldehyde	Pale green to Brown colour produced	+	Cinnamaldehyde
2.	Curcuma longa	Alkaloid test Conc. Sulphuric acid	Yellow to dark brown Yellow to red	+ −	Alkaloids
3.	Mentha	Alkaloid test	Brown to yellow	+	Alkaloids
4.	Allirum sativum	Flavonoid Test for allicin	Yellow to colourless	+	Allicin
5.	Ocimum sanctum	Flavonoid test	Yellow to colourless	+	Flavonoids
6.	Azadirachta indica	Flavonoid test for estradiols	Yellow to colourless	+	Estradiols
7.	Caseria esculanta	Alkaloid test for diterpenoids	Brown to yellow	+	Alkaloids

5.0 Conclusions

Plants are very useful source of various bioactive compounds which have direct or indirect use in the treatment of various human ailments. From the time immemorial, human civilizations have been exploring and using various plants and plant products to cure the deadly diseases. Therefore this study focuses in extraction and identification active components from herbs. The identified components were cinnamaldehyde, allicin, estradiol and diterpenoids, alkaloids and flavonoids these components will be further applied in the form of herbal medicine against prevention of polycystic ovary syndrome.

References

[1] Hadi A, Moradi S, Ghavami A, Khalesi S, Kafeshani M (2020). Effect of probiotics and synbiotics on selected anthropometric and biochemical measures in women with polycystic ovary syndrome: a systematic review and meta-analysis. European Journal of Clinical Nutrition. 74: 543-547. https://doi. org/10.1038/s41430-019-0434-9.

[2] Ybarra M, Franco RR, Cominato L, Sampaio RB, Sucena da Rocha SM, Damiani D (2018). Polycystic Ovary syndrome among obese adolescents. J Gynecol Endocrinol. 34: 45–48.

[3] Ding T, Hardiman PJ, Petersen I, Wang FF, Qu F, Baio G (2017). The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: a systematic review and meta-analysis. Oncotarget. 8: 96351–96358.

[4] Naz1 M, Tehrani F, Hamid A, Fazlollah A, Giti O, Farzaneh R, Vida G. The prevalence of polycystic ovary syndrome in adolescents: A systematic review and meta-analysis International Journal of Reproductive BioMedicine, 17, Issue no. 8.

[5] Rashidi H, Tehrani F, Bahri M, Tohidi M, Azizi F. To what extent does the use of the Rotterdam criteria affect the prevalence of polycystic ovary syndrome? A community-based study from the Southwest of Iran. Eur J Obstet Gynecol Reprod Biol 2014; 174: 100–105.

[6] Ganie M, Vishnu V, Imtiyaz A, Mohammad S, Tasleem A & Aafia R. Epidemiology, pathogenesis, genetics & management of polycystic ovary syndrome in India Indian J Med Res 150, October 2019, pp 333-344.

[7] Dr. Padmalaya Rath A case of infertility due to PCOS treated successfully with homoeo pathy International Journal of Homoeo pathic Sciences 2020; 4(2): 249-260.

[8] Misra, A.; Pachauree, S. (2002) Retention Behavior of Metal Ions on Calcium Sulphate Layers: Separation of Mercury. Ind. J. Chem. Technol., 9, 239-244.

[9] Rakhi A, Jai R. Screening for Mercury in Aqueous Environmental Samples and Urine Samples using Thin Layer Chromatography, 79, 2457 (2007).

[10] Bhatia S, Kalpana N, Tanmoy B, Ajay S, Kiran S.Evaluation of pharmacognostical, phytochemical and anti-microbial properties of Porphyra vietnamensisDOI: 10.4103/0973-8258.155065.

[11] Sanguri S, Kapil S, Gopinathan P, et al. Comparative screening of antibacterial and antifungal activities of some Weeds and medicinal plants leaf extracts: An in-vitro study. Elixir Applied Botany. 2012; 47: 8903-8905.

[12] Paul S and Sainsburg D. Dictionary of Microbiology and Molecular Biology. 2nd Edition. John Wiley and Sons Publishers Brisbane. 1996; 46.

[13] Thapar P and Garcha S. Incidence and characterization of Pseudomonas isolated from spoiled fresh produce. Indian Journal of Experimental Biology. 2017; 55: 372-376.

[14] Bashir A, Sayed El H, Amiri HM. Antimicrobial activity of certain plants used in the folk medicine of United Arab Emirates. Fitoterapia. 1992; 4: 371-375.

[15] Marka R, Samatha T, Srinivas P, Shyamsundarachary R, Rama S. N.* Preliminary Phytochemical Analysis of Leaf, Stem, Root and Seed Extracts of Arachis hypogaea L. Int. J. Pharm. Sci. Rev. Res., 20(1), May-june 2013.