Research article

Rapports De Pharmacie Vol.7 (3), 2021, 57-63
ISSN: 2455-0507

PHYTOCHEMICAL SCREENING AND ANTI ACNE EFFECT OF CLITORIA TERNATEA

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ABSTRACT

Objective: To study the anti-acne effect of *Clitoria ternatea* flower extracts on *Propionibacterium acnes* and to identify photochemical constituents of *Clitoria ternatea* flower extracts.

Method: The extraction method used in this experiment was Soxhlet extraction using methanol, distilled water and hexane. Phytochemical screening to determine the present ofreducingsugar, starch, protein, volatileoil, steroid, cardiacglycoside, anthraquinoneglycoside, flavonoids, alkaloid, phenol, saponin and tannin in the extracts of *Clitoria ternatea* flower were carried out. Anti-acne effect test on *P. acnes* were carried out by using agarwell diffusion test. **Result**: The phytochemical constituents present in methanol and aqueous extract of *Clitoria ternatea* flower were: carbohydrate, reducing sugar, starch, protein, steroid, anthraquinone glycoside, flavonoids, saponin and tannin, whereas only volatile oil was identified in hexane extract. Agar well diffusion test on *P.acnes* showed that methanol and aqueous extract of *Clitoria ternatea* showing promising anti acne effect. **Conclusion**: Various bioactive constituents present in the flower of *Clitoria ternatea* were believed to exhibit the antimicrobial properties of *Clitoria ternatea* flowers against possible acne-causing pathogen, *P.acnes*.

Keywords: *P.acnes*, disc diffusion test, bioactive constituents

INTRODUCTION

Acne is a common skin disease, which affects majority of individual in different age group [1].Acne produces emotional scars that last lifelong which causes negative effects in individual's confidence. Bacterial resistance towards antibiotic urges the search for new lead molecule to combat acne [2]. Therefore, medicinal plants can be explored to be used as remedies to prevent and cure acne.

There are various ethnobot any uses of *Clitoria ternatea.* For instance, in view of herb and spiceuses, a blue, edible dye is extracted from its flowers in order to make traditional Malay pastries.In addition, in view of medicinal uses; people use its roots to make into powder that treat illnesssuch as sore throats, abdominal swelling or mucus disorders [3]. Mixture of juice from the roots andcold milk serves as a traditional remedy to resolve chronic bronchitis or remove phlegm [4].

MATERIALS AND METHODOLOGY

Plant materials:

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Flowers of *Clitoria ternatea* were purchased in Miri, Sarawak. Flowers were separated from the stem and dried in hot air oven for72 hours at 45°*C.*

Extraction:

The dried flowers were grinded into powder by using laboratory blender. 80gmof powder was then subjected to successive hot continuous Soxhlet extraction by using hexane, methanol and water as three different solvents. Hexane and methanol were used for obtaining organic extracts, where as water was used for obtaining aqueous extracts. After the extraction process, solventswereevaporatedoutfromtheextracts by heating on hot water bath for total 30 hours untilitbecame a concentrated paste. The extraction yield was calculated by following formulae:

$$\frac{Netweightofextractsobtained(z)}{WeightofClitoriaternateapowder usedfortheextraction} X\,100$$

Phytochemical screening of Clitoria ternatea extract:

Clitoria ternatea extracts of 2mg/ml were used by diluting the net yield with0.1%DMSO solution. The procedures for each test were stated below inTable3 [11].

Table1:Procedures and indications for photochemical tests on *Clitoria ternatea* extracts

Constituents	Test	Procedure	Indications
Carbohydrates	Molisch's test	2ml of Molisch's reagent into was added to 3ml of extract, and the mixture was shook.2ml of concentrated sulfuric acid was poured carefully down the side of test tube.	Formation of a red /dull violet colour at the inter-phaseof the two layers indicates presence of carbohydrates.
Reducingsugars	Fehling'stest	Equal quantity of Fehling'sA and B solution was addedinto the extract. The mixturewas heated in boiling waterbathfor10 minutes.	Brick red precipitateindicates the presenceofreducingsugars.
	Benedict'stest	1mlofBenedict'sreagentwasaddedinto1mlofextract.Themixturewasheated in boiling water bathfor5 minutes.	Brick red precipitateindicates the presenceofreducingsugars.
Starch	Iodinetest	Fewdropsofiodinewereaddedtotheextract.	Blue-black colour indicates presence of starch.
Protein	Million'stest	Few drops of Million'sreagent were added to the extract.	White precipitate indicates the presence of protein and free aminoacids.

Constituents	Test	Procedure	Indications
Flavonoids	Shinoda test	In a test tube containing 0.5ml of the extract,5-10drops of dilute HCl followed by a small piece of magnesium were added. The solution was boiled for afew minutes.	A pink, reddish pinkorbrowncolorindicatespresenceofflavonoids.

Alkaloid	Wagner'stest	1ml of extract was acidifiedwith1.5%of Hal, a few drops of Wagner's reagent were added.	A brown precipitateindicatespresenceofalkaloids.
Phenols	FeCl3 test	2ml of distilled water and afewdropsof10%ferricchloride solution were addedintoasmallquantityofextract.	Ablueorgreencolorisproducedindicates the presenceofphenols.
Saponin	Frothtest	5mlofdistilledwaterwasaddedintotheextract,the mixture was shake vigorously and left for 3min.	Honeycomblikefrothindicatesthepresenceof saponins.
Tannin	Braemer's test	Fewdropsof5%aqueousferric chloride solution wasaddedinto theextract.	A bluish black colorindicates the presenceoftannins.

Antimicrobial activity:

The whole process was carried out in an aseptic environment under a laminar air flow cabinet. Firstly,100µl of *P.acnes* liquid bacteria culture was pipette on to the agar plate by using a micropipette.AL-shaped glass spreader was used to evenly spread the bacteria culture on the agar plate, and the liquid bacteria was left to dry up. After that, five wells of diameter7mm were made by punching the autoclaved micropipette tips on to the agar. Each well was labeled as: a, b, c, +ve control and-ve control. They were filled respectively with 100µl of differentsolution by using micropipette.The plates were incubated anaerobically upwards at 37 °C for 36 h, and finally, the zones of inhibition were measured and recorded for each extract [12].

RESULTS AND DISCUSSION:

The extraction yield for *Clitoreaterna tea* different extract was depicted in table 2. The highest extraction yield was obtained for methanol and water extract. This indicates that polar solvents able to extract high yield of *Clitoreaterna tea* extract as compared to hexane which is having less polarity.

Table2: Extract ion percentage yield of *Clitoria ternatea* extracts

Extracts	Extractionpercentageyield, w/w (%)
Methanol	46.58
Hexane	1.11
Water	44.93

Photochemical tests were carried out on methanol, hexane and water extracts of

*Clitoriaternatea*flower.Theresultsofthequalitativephytochemicalscreeningindicates thepresenceofcarbohydrate,reducingsugar,starch,protein,volatileoil,steroid,cardiacglycoside,anthraquinone glycoside,flavonoids,alkaloid,phenol,saponinandtanninintheextractsof*Clitoriaternatea*flower(Table3).

Table3: Results of phytochemical test carried out on *Clitoria ternatea* extracts

PhytochemicalTest		Extracts of *Clitoria ternatea* flower (Different solvent)		
Presenceof Metabolites	NameofTest	Methanol	Distilledwater	Hexane
1.Carbohydrate	Molisch's Test	+	+	–
2.Reducing Sugar	Fehling'sTest	+	+	–
	Benedict'stest	–	+	–
3.Starch	IodineTest	+	+	–
4.Protein	Million'sTest	+	+	–
	Ninhydrin Test	+	+	–
	BiuretTest	+	–	–
5.Volatileoil	FilterpaperTest	–	–	+
	SolubilityTest	–	–	+
6.Steroid	Salkowski reaction	+	+	–
7.Cardiac glycoside	Legal's Test	–	–	–
	Keller-Kiliani Test	–	–	–
8.Anthraquinone glycoside	Borntrager's test	+	+	–
9.Flavonoids	Shinoda test	+	+	–
10.Alkaloid	Wagner'sTest	–	–	–
11.Phenol	$FeCl_3$ Test	–	–	–

12.Saponin	FrothTest	+	+	–
13.Tannin	Braemer's test	+	+	–

"+ve" indicates presence and"-ve"indicates absence.

Table4: Test results of agar well diffusion test on *P. acnes*

Clitoria ternatea flower extracts	Zoneofinhibitionofdifferentwells,ZOI(mm)	
	Plates Wells	1
1.Methanol	a	8.5
	b	7.5
	c	8.5
	-ve control	0
2.Distilledwater	a	0
	b	7.5
	c	8.0
	-ve control	0

a = 1.0g/mlb = 2.5g/mlc=5. 0g/ml
-ve control =0.5%DMSO

According to the result btained, carbohydrate, reducing sugar, starch, protein, steroid, anthraquinone glycoside, flavonoids, saponin and tannin were present in both methanol and water extract of *Clitoria ternatea*. On the other hand, only volatile oil was identified in the hexane extract of *Clitoria ternatea*. This might be due to the different polarities of the extraction solvents used. The polarity increased in a sequence of: Hexane>Methanol >Water. Hexane is a non-polar solvent, thus it played Darleen extracting non-polar constituents such as volatile oiling *Clitoria ternatea*. Methanol was a suitable organic solvent for extraction of most primary and secondary metabolites, and most importantly it was a polar solvent that could extract hydrophilic compounds such as flavonoids, tannin and phenol [13].Cardiac glycoside, alkaloid and phenol were absent in all the *Clitoria ternatea* flower extracts. However, these chemical constituents were present in *Clitoriaternatea* leaf extracts, seed and root [14]. This showed that different parts of *Clitoria ternatea* consist of different bioactive constituents. Based on the result obtained, there were some similarities and differences when compared to phytochemical tests done by other researches previously [15-16].

AccordingtoresearchcarriedoutbyLinggametalandKumaretal,tanninswereabsentinmethanolandaqueousextractsof*Clitoriaternatea*flower; whereas, based on this current research, positive result were obtained for tannins in both methanol and aqueous extracts of *Clitoriaternatea* flower. In addition, positive result on saponin and steroid was obtained in this current research which was supported by researches carried out by Kumar et al. Furthermore, according to phytochemical tests carried out by Linggametal and Nallaetal, anthraquinone glycoside was absent in *Clitoriaternatea* flower; whereas based on this result, anthraquinone glycoside was present in both methanol and aqueous extracts of *Clitoria ternatea* flower. In short, these dissimilarities may be due to thedifferences in extraction method/ extraction solvent/ extracts concentration/ or phytochemicaltestprocedures applied during eachresearch.

Based on these obtained results, the presence of steroid, anthraquinone glycoside, flavonoids, saponin and tannin in *Clitoria ternatea* flower might be the contributor of its antibacterial effect (Table 4). According to other literature reviews, the flavonol glycoside present in roots wasreported to have antibacterial activity [17]. The presence of flavonoids might contribute to the antibacterial effect of *Clitoria ternatea* flower extracts as well. In addition, tannin also processed unique antiviral as well as antibacterialpropertieswhichmadeitapotentchemicalconstituentexpressingantibioticeffects [18].Numerousinvitroorinvivoantimicrobialactivitiesofanthraquinoneswerereported that showed it as a potential antimicrobial agent [19].

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