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STANDARDIZATION AND ANTIBACTERIAL SCREENING OF *OCIMUM BASILICUM* (LAMIACEAE) LEAF, SEED AND STEM EXTRACTS AGAINST THE ORGANISM OF *PROPIONIBACTERIUM ACNE*S

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Abstract

Propionibacterium acne bacteria live deep within follicles and pores, away from the surface of the skin. In these follicles, *P. acnes* bacteria use sebum, cellular debris and metabolic byproducts from the surrounding skin tissue as their primary sources of energy and nutrients. Elevated production of sebum by hyperactive sebaceous glands (sebaceous hyperplasia) or blockage of the follicle can cause *P. acnes* bacteria to grow and multiply. The Medicinal herb of *Ocimum basilicum* (Lamiaceae) leaf, seed and stem was screened for its Macroscopical, Physiochemical parameters, Florescence analysis (Day light, and long UV), The dried leaf powder (15gms) was extracted by Cold maceration with 70% alcohol. The percentage value of extracts is 7.38. The dried seed and stem powder material 25gms was subjected to soxhlet extraction with 99% ethanol for continuous hot extraction for 4 hours separately. The extracts were concentrated under reduced pressure to obtain the extract solid restudies. The percentage value of the extract was 19.75% and 8.375. These extracts were qualitatively screened. The qualitative chemical analysis is made by based upon the color reaction; Alkaloids, Amino acids, flavonoids, Phenolic groups, saponins, and Tannins were commonly present in each extract of *Ocimum basilicum*. Further confirmed by thin layer chromatography also performed depending upon the mobile phase and detecting agents were used. The plant of Ocimum basilicum 70% ethanolic leaf extract, 99% ethanolic seed and stem extracts effective against the Micro organisms of *propionibacterium acne* by disc diffusion method. The extract of leaf, seed and stem of O*cimum basilicum* showed highest activity at minimum concentration. The Plant extracts having marked activity against the microorganism tested in dose dependent manner.

Keywords: *Ocimum basilicum, Propionibacterium acne,* Flavonoids, Soxhlet, Disc diffusion.

Introduction

The Plant Ocimum basilicum (Lamiaceae) is called as sweet basil, Basil is originally native to India and other tropical regions of Asia, having been cultivated there for more than 5,000 years. The word *basil* comes from the Greek (*basileus*), meaning "king", as it has come to be associated with the Feast of the Cross commemorating the finding of the True Cross by St Helena mother of the emperor St. Constantine. Alternatively the herbalist John Gerard noted of basil that those

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stung by scorpions would feel no pain if they ate of basil.

P. acnes bacteria live deep within follicles and pores, away from the surface of the skin. In these follicles, *P. acnes* bacteria use sebum, cellular debris and metabolic byproducts from the surrounding skin tissue as their primary sources of energy and nutrients. Elevated production of sebum by hyperactive sebaceous glands (sebaceous hyperplasia) or blockage of the follicle can cause *P. acnes* bacteria to grow and multiply.

P. acnes[2] bacteria secrete many proteins, including several digestive enzymes. These enzymes are involved in the digestion of sebum and the acquisition of other nutrients. They can also destabilize the layers of cells that form the walls of the follicle. The cellular damage, metabolic byproducts and bacterial debris produced by the rapid growth of *P. acnes*[3] in follicles can trigger inflammation. This inflammation can lead to the symptoms associated with some common skin disorders, such as folliculitis and acne vulgaris. In the present study, an attempt has been made to enrich the knowledge of antibacterial activity Ocimum basilicum (Leaf, Stem, seed) Ethanolic extract against *P.acnes in skin diseases of pimples.*

Materials and methods

Plant material

The plants of *Ocimum basilicum*[4] *was* collected from Thirumalaisamudram 7km away from Thanjavur (Tamil Nadu) in the month of December 2011. The plants was identified by local people of that village and authenticated by Dr. N.Ravichandran, Asst. Professor, Drug Testing Laboratory, CARISM, SASTRA University Thanjavur, and the Voucher specimen is preserved in laboratory for future reference.

Chemicals

All the reagents used were of analytical grade obtained from S.D. fine chemicals, Ltd, and Hi Media, Mumbai.

Macroscopic Characters of *Ocimum basilicum Leaf*:

The Macroscopic evaluation was carried out for shape, size, color, odor, taste and fracture of the drug.

Physio-chemical screeing of *Ocimum basilicum leaf,stem,seed:*

Different physio-chemical values such as Ash value, extractive values, loss on drying, foreign organic matter, Crude fiber content, were determined.

Fluorescence analysis study of *Ocimum basilicum* leaf, seed and stem powder

Fluorescence analysis study of powdered drug material with different reagents was carried out to observe the color reactions.

Preparation of Extracts from *Ocimum basilicum* leaf, seed, stem:

The leaves, stem, seed were dried under shade, powdered and passed through 40 meshes and stored in closed vessel separately for further use. The dried leaf powder (15gms) was extracted by Cold maceration with 70% alcohol. The percentage value of extracts is 7.38. The dried seed and stem powder material 25gms was subjected to soxhlet extraction with 99% ethanol for continous hot extraction for 4 hours separately. The extracts were concentrated under reduced pressure to obtain the extract solid restudies. The percentage value of the extract was 19.75%. 8.375.

Phytochemical Evaluation of *Ocimum basilicum* leaf, seed and stem extracts

The Ethanolic Extract of *Ocimum basilicum* leaf, seed and stem *subjected* to preliminary Phytochemical tests followed by the methods of Harbone (1998), and Trease and Evans (1983) and the phytoconstituents reported in table.

Screening of Thin layer Chromatography:

TLC for Alkaloids

Stationary phase : Silicagel G
Mobile Phase :Butanol:Aceticacid:Water (4:5:1)
:Chloroform:Methonol:Amonia (8:4:1.5)
:Chloroform:Diethylamine(9:1)
:Toluene:Ethylacetate:Diethyl amine(7:2:1)
Detecting Reagent : Dragendorffs reagent.

TLC for Terpenes:

Stationary Phase :Silicagel G
Mobile Phase :Hexane:acetone (9:1)
:Ethylacetate:Toluene:Formic acid(5:5:1.5)
:Toluene:Chloroform:Ethyl alcohol(4.5:4.5:1)
:Ethylacetate:Aceticacid:Formic acid:water(10:1:1:1)
Detecting Reagent: Iodine Chamber

TLC for Saponins:
Stationary Phase :SilicagelG
Mobile Phase
:Chloroform:Methnol:Water (7:4:1)
:Chloroform:Aceticacid:Methanol:water(6.4:3.2:1.2:0.8)
:Ethylacetate:Ethylalcohol:Water:ammonia
(6.5:2.5:0.9:0.1)
:Ethylacetate:Methanol(9.7:0.3)
Detecting Reagent: Iodine Chamber

TLC for Flavonoids:
Stationary Phase :SilicagelG
Mobile Phase :Chloroform:Ethylacetate(6:4)
:Toluene:Ethylacetate:Formic acid(5:4:1)
:Toluene:Ethyl acetate(9.5:0.5)
Detecting Reagent: Iodine chamber

TLC for Phenolic compounds:
Stationary Phase :SilicagelG
Mobile phase :Butane-2-ol:acetic acid:water (14:1:5)
:Toluene:acetone:formic acid (60:60:10)
Detecting Reagent: Ammonia vapour

Determination of Antibacterial Activity of crude extract of *Ocimum basilicum* (Leaf, seed and stem)[5]

Test Microorganisms

All the microbial strains of human pathogens used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. *Propionibacterium acnes (*MTCC 1951) strain was used for the present study.

Selective antibiotics and media

Selective standard antibiotic drug (10µg/ml) was used against organism for Eg.Clindamycyin against *Propionibacterium acnes* respectively. Nutrient Agar (NA) was used respectively for testing the antibacterial activity.

Determination of Antibacterial Activity of Ethanolic crude extract of *Ocimum basilicum* (Leaf, Seed and Stem) by disc diffusion method:

The in vitro antibacterial activity Muller Hinton Agar plates were seeded with 24 h broth culture of different bacteria. In each of these plates, the various extracts were loaded (50, 100, 200) were loaded on to different filter paper discs prepared from What man No: 1 Filter paper. The discs were then placed on agar medium containing the cultures and incubated for 24hours at 37^0c. The diameter of zone of growth inhibition was recorded. The effects with were compared with that of standard antibiotic clindamycin (10mcg/discs) and 70% alcohol served as control.

Results and discussions

Macroscopical Characters of *Ocimum basilicum* leaf

Fig. 01: *Ocimum basilicum* leaf

Colour: Pale green to dark green, Odour: Aromatic, Taste: Pungent, Size: Length 2.5 cm to 3.6cm Width 1.5cm to 2.0 cm, Shape: Ovate lanceolate, entire margin , petiole present small spine present, reticulate veination, Fracture: Short fracture.

Macroscopical Characters of *Ocimum basilicum* seed

Fig. 02: *Ocimum basilicum* seed

Colour: dark brown to blackish, Odour: Characteristic, Taste: pungent, Size: 0.3 to 0.4 mm length, 0.2 to 0.3 mm width.

Macroscopical characters of Ocimum basilicum stem

Fig. 03: Ocimum basilicum stem

Colour: Pale green to dark green, odour: Aromatic, Taste: pungent, size: depends upon the age of the plant size should be vary, shape: cylindrical, Fracture: short, pubescent present.

Table No. 01: Physiochemical Parameters of *Ocimum* Leaf powder

S.No	Parameters	*Ocimum basilicum* leaf powder %W/W	*Ocimum basilicum* seed powder %W/W	*Ocimum basilicum* Stem powder %W/W
1.	Pet ether Soluble extractive	8%	4%	8%
2.	Chloroform Soluble extractive	24%	24%	8%
3.	Acetone soluble extractive	8%	8%	5.6%
4.	Ethanol soluble extractive	20%	16%	8%
5.	Methanol Soluble extractive	24%	28%	5.8%
8.	Water soluble extractive	21%	20%	14%
9.	Foreign organic matter	1.8%	4%	4%
10.	Loss on drying	1.2%	3.4%	3.4%
11.	Crude fiber content	4.5%	4%	4%
12.	Total Ash	4%	4.3%	5%
13.	Acid insoluble ash	1.2%	2%	1.75%
14.	Sulphated ash	5%	5.3%	6%

Table No. 02: Fluorescence analysis studies of *Ocimum basilicum* Leaf powder

S.No	Treatment for Leaf powder	Leaf Powder		Seed Powder		Stem powder	
		Visible light	Long UV 365nm	Visible light	Long UV 365nm	Visible light	Long UV 365nm
1.	Powder	Pale green	Dark green	Pale greenish brown	Pale brown	Pale greenish brown	Pale brown
2.	Powder + 50%of H_2so_4	Pale brown	Dark green	Pale green	Brownish green	Pale brown	Greenish brown
3.	Powder + 10% of NaoH (Aqueous)	Yellowish brown	Green	brown	Pale greenish to brown	Yellowish brown	Pale green
4.	Powder + 10% of NaoH (Alcoholic)	Dark brown	Dark green	Dark brown	Yellowish	Yellowish brown	Green
5.	Hexane Extract	green	Pale brown	Pale brown	Pink	Yellowish green	Pale brown
6.	Benzene Extract	Pale green	Pink	Greenish brown	Orange	Pale brown	Pale green
7.	Acetone Extract	Dark green	Pale green	Light green	Pink	Yellowish brown	Pale green
8.	Alcoholic extract	Pale green	Green	Pale brown	Green	Pale brown	Pale brown
9.	Aqueous Extract	brown	Olive green	Pale brown	green	Pale yellow	Pale green

Table No. 03: Preliminary Phytochemical screening of the Ethanolic extracts of *Ocimum basilicum (Leaf, seed and stem)*

Phytoconstituents	*Ocimum basilicum (Leaf)*	*Ocimum basilicum (Seed)*	*Ocimum basilicum (Stem)*
Alkaloids	+	+	+
Aminoacids	+	+	+
Carbohydrates	-	-	-
Glycosides	-	-	-
Flavonoids	+	+	+
Phenolic groups	+	-	-
Fats and oils	-	+	-
Saponins	+	+	+
Tannins	+	-	-
Proteins	+	+	-

+ Present, - Absent

Table No. 04: screening of Thin layer Chromatography of Ethanolic extract of *Ocimum basilicum* leaf, seed and stem

Name of Phytoconstituents	*(Leaf) Rf values*	*(seed) Rf values*	*(stem) Rf values*
Alkaloids	0.75 0.375	0.375 0.66	0.47 058
Saponins	0.46	0.41 0.54	0.4 0.6
Terpenes	0.52	0.7	0.68

Table No. 05: Determination of Antimicrobial Activity of ethanolic leaf extracts of *Ocimum basilicum* (Leaf, seed and stem)

Name of Micro organisms	Zone of inhibition in mm										
	Leaf extract			Seed extract			Stem Extract			C	Std
	200 mg	100 mg	50 mg	200 mg	100 mg	50 mg	200 mg	100 mg	50 mg		30mcg/ml
***Propioni bacterium acnes* (MTCC 1951)**	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0	0	0. 5 ± 0.1

C-Control std-Standard

The extract of leaf, seed and stem of O*cimum basilicum* showed highest activity at minimum concentration. The Plant extracts having marked activity against the microorganism tested in dose dependent manner.

Conclusion

The Medicinal herb of *Ocimum basilicum* (Lamiaceae) leaf, seed and stem was screened for its Macroscopical, Physiochemical parameters, Florescence analysis (Day light, and long UV), The dried leaf powder (15gms) was extracted by Cold maceration with 70% alcohol. The percentage value of extracts is 7.38. The dried seed and stem powder material 25gms was subjected to soxhlet extraction with 99% ethanol for continuous hot extraction for 4 hours separately. The extracts were concentrated under reduced pressure to obtain the extract solid restudies. The percentage value of the extract was 19.75% and 8.375. These extracts were qualitatively screened. The qualitative chemical analysis is made by based upon the color reaction; Alkaloids, Amino acids, flavonoids, Phenolic groups, saponins, and Tannins were commonly present in each extract of *Ocimum basilicum*.[6] Further confirmed by thin layer chromatography also performed depending upon the mobile phase and detecting agents were used. The plant of Ocimum basilicum 70% ethanolic leaf extract, 99% ethanolic seed and stem extracts effective against the Micro organisms of *propionibacterium acne* by disc diffusion method.

The extract of leaf, seed and stem of O*cimum basilicum*[7] showed highest activity at minimum concentration. The Plant extracts having marked activity against the microorganism tested in dose dependent manner.

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