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Antiinflammatory and antimicrobial activities of Thai plant extracts for hemorrhoid treatment

Suchada Kittisrisopit[1*], Sumalee Parnthong[2] , Arunporn Itharat[2**]

[1]Student of Master Degree of Medical Sciences Program (Applied Thai Traditional Medicine) Faculty of Medicine, Thammasat University
[2]Applied Thai Traditional Medicine Centre, Faculty of Medicine, Thammasat University, Thailand.
* Presenting author, ** Corresponding author

Abstract

The objective of this research was investigating anti-inflammatory and antimicrobial activities of Thai medicinal plants used to treat hemorrhoid. Inhibitory activity against LPS induced NO production in RAW 264.7 cell lines was used as assay for anti-inflammation. Disc diffusion method and MIC values were evaluated for antimicrobial against gram positive (*Staphylloccus aureus* and *Bacillus subtilis*), gram negative (*Escherichia coli*) and fungi or yeast (*Candida albican*). The extraction method, similar to folk doctors, used maceration in ethanol and boiling in water. Five plants: *Anacyclus pyrethrum* (L.) DC., *Angelica sylvestris* Linn., *Artemisia vulgaris* Linn., *Terminalia chebula* Retz gall and *Picrorrhiza kurroa* Royle. Ex Benth. in hemorrhoid preparation were selected to investigate. The results were found that *Artemisia vulgaris* exhibited the most potent anti-inflammatory activity with an IC_{50} value of 28.09 μg/ml and *Terminalia chebula* Retz gall extract showed the highest antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Candida albicans* with concentration of 0.28 mg/ml, 5 mg/ml and 1 mg/ml respectively.

Keywords: antiinflammatory, nitric oxide, antimicrobial, Thai traditional medicine for hemorrhoid

Introduction

Anacyclus pyrethrum (L.) DC. (AP), *Angelica sylvestris* Linn. (AS), *Artemisia vulgaris* Linn. (AV), *Terminalia chebula* Retz gall (TG) and *Picrorrhiza kurroa* Royle. Ex Benth. (PK) are commonly used for the many formula of Thai traditional medicine and they are ingredients in hemorrhoid preparation. Thus, the investigation of this research focused at antiinflammatory and antimicrobial activities for supporting using these plant in hemorrhoid preparation. The inhibitory against LPS induced Nitric Oxide production in RAW 264.7 cell lines was used as preliminary study of these plant extracts for antiinflammation activity because of nitric oxide (NO) is one of the inflammatory mediators causing inflammation in many organs and microorganisms cause diverse biological effects. There is no previous report on the antiinflammatory activities of these plants. In addition to antimicrobial was also studied with these plant extracts for hemorrhoid treatment. These results should be used for supporting using these plants as ingredients in hemorrhoid preparation.

Materials and methods

Plant materials

A. sylvestris, *A. pyrethrum*, *A. vulgaris*, *P. kurroa* and *T.chebula* gall were purchased from the Thai herb shop . Plant materials were dried and powdered. The powder was macerated three times with 95% ethanol for 3 days each. The extracts were concentrated under reduced pressure by rotary evaporator.

Antiinflamatory activity by nitric oxide production in RAW 264.7[1]

The murine macrophage cells (Raw 264.7) were cultured in complete medium and incubate at 37 °C in 5% CO_2 unless otherwise stated. The extracts were prepared at a concentration of 10 mg/ml in DMSO as stock solution. After 70–80% confluence, the cells were treated with LPS (10 µg/ml) and extracts were diluted as concentration 3, 10, 30 and 100 µg/ml. The nitric oxide production was determined at 540 nm. Briefly, 100 µl supernatant of samples were pipetted in 96 well plate and put an equal volume of Griess reagent (0.1% naphthalene diamine dihydrochloride, 1% sulfanilamide in 5% H_2SO_4)in that 96 well plate and incubated for 10 min at room temperature. The IC_{50} calculated using Prism program. Cytotoxicity testing was also tested to determine that nitric oxide production was not produced by destroy cell membrane. This testing used MTT assay or the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric method. The absorbance was determined at 540 nm.

Antimicrobial activity[2,3]

The antimicrobial activities of all plant extracts were determined against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028). The disc diffusion assay (Kirby-Bauer Method) was used to screen for all extracts. Minimal inhibitory concentration was determined as follows: the plant extracts were dissolved in DMSO. The MHB solution was brought for preparing plant extracts to be concentration ranges of 0.156 – 5.0 mg/ml. 100 µl of each concentration was added in a well (96-well plate) containing of 100 µl of MHB and inoculums. All mixtures were incubated at 37°C for 24 hours. MIC values of the plant extracts against bacterial strains were evaluated by resazuin as this minimum dilution after overnight incubation .

Results and Discussion

All ethanolic extracts showed inhibition NO production at concentration 100µg/ml (AP, AS, AV, PK and TC are 49.55, 90.01, 88.71, 73.75 and 90.73 µg/ml respectively), only two extracts such as AV and TG had no cytotoxicity against RAW 256.7 cells and can inhibit NO oxide production more than 50% . They were dilution and evaluated by inhibition nitric oxide production and found that the IC_{50} (n=3) of AV and TG were 28.09±7.02 and 41.94±9.43 µg/ml respectively .It is concluded that AV showed the best inhibitory effect on NO production activity. It can possess that it should be antiinflammatory of hemorrhoid product.

Table 1 Antiinflamatory effect of ethanolic extracts of plants at concentration 100 µg/ml evaluated by % inhibition of Nitric Oxide (NO) production

Extract	Code	%inhibition of NO production (%cytotoxic)				IC_{50} (µg/ml)
		3 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml	
Anacyclus pyrethrum(L.)DC	AP	1.0 (2.0)	3.1 (3.7)	15.5 (3.9)	49.6 (9.1)	-
Angelica sylvestris Linn.	AS	2.2 (13.3)	12.3 (22.6)	35.1 (28.5)	90.0 (46.7)	-
Artemisia vulgaris Linn.	AV	4.8±16.4 (6.7±3.4)	16.4±6.6 (8.9±3.4)	57.2±15.0 (11.6±3.3)	88.7±4.7 (7.7±6.9)	28.09±7.02
Picrorrhiza kurroa Royle. Ex Benth.	PK	0.0 (23.9)	4.5 (42.2)	20.7 (32.9)	73.5 (50.1)	-
Terminalia chebula Retz gall	TG	4.3±1.5 (10.9±6.5)	13.3±3.5 (8.8±2.4)	39.6±6.0 (15.0±6.2)	90.7± 1.8 (16.9±1.4)	41.94±9.43

In the table 2, *Terminalia* gall extract exhibited antimicrobial activity against microorganisms such as *S.aureus, B. subtilis* and *C. albicans.* MIC values were found 281.25 µg/ml 5 mg/ml and 1 mg/ml respectively. It related with the previous report because it contained tannin which can inhibit microbial . *A. sylvestris* can inhibit *S. aureus* and *B. subtilis* (MIC =2.5 , 1 mg/ml respectively). Whereas *P. kurroa* was against *S. aureus* (4.5 mg/ml) and there is no antimicrobial activity from *A. vulgaris* and *A. pyrethrum* extracts.

Table 2 antimicrobial activity of ethanolic extracts ; *A. sylvestris* (AS), *A. pyrethrum* (AP), *A. vulgaris* (AV), *P. kurroa* (PK) and *Terminalia* gall extract (TG) (n=3)

Extract	Inhibition zone(mm.) and MIC (n=3)			
	S.aureus ATCC 25923	*B. subtilis* ATCC 6633	*E.coli* ATCC 25922	*C. albicans* ATCC 90028
AP	-	-	-	-
AS	7.33 ± 0.58 2.5 mg/ml	7.33 ± 0.58 1 mg/ml	-	-
AV	-	-	-	-
PK	11.67 ± 0.58 4.5 mg/ml	8.33 ± 0.58 > 5 mg/ml	-	
TG	16.68 + 0.58 281.25 µg/ml	9.33 + 2.31 5 mg/ml	-	19.33 + 4.04 1 mg/ml

Conclusion

From these results were concluded that the ethanolic extracts *Artemisia vulgaris* was ingredients for antiinflammation of hemorrhoid preparation. *Terminalia chebula* gall was ingredient for antimicrobial activity especially gram positive bacteria cause of wound because of tannin containing in this gall. These results can support using these five plant for relief pain from inflammation and wound from hemorrhoid. However , it should be confirm by another antiinflammation assay such as COXII inhibitor and test all of these plants *in vivo.*

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