NOTE

# **Evaluation of anti-acne activity of selected Sudanese medicinal plants**

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**Abstract** Acne vulgaris is the most common skin disease in the world, and the number of antibiotics resistant to acne-inducing bacterial strains has been increasing in the past years. Natural substances from plants are promising candidates to treat this disease. The methanol and 50 % (v/v) ethanol extracts of 29 plant species traditionally used in Sudan for treatment of a variety of diseases were tested in vitro for their potential anti-acne activity. The activities of these extracts were determined using an antibacterial assay against Propionibacterium acnes, a lipase inhibitory assay, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay. The results showed that methanol and 50 % ethanol extracts of Terminalia laxiflora Engl & Diels wood exhibited good antibacterial activity (minimum inhibitory concentration 0.13 mg/ml). The 50 % ethanol extracts of Abrus precatorius L. seed, T. laxiflora Engl & Diels and methanol extract of Acacia nilotica (L.) pods showed lipase inhibitory activity more than 70 % at 500 µg/ml. The methanol extracts of A. nilotica (L.) pods showed the best DPPH radical scavenging activity ( $IC_{50}$ ) 1.32 µg/ml). Total phenolic, flavonoid and total tannin contents of selected plant extracts shown anti-acne activities were investigated. Almost all selected extracts contained phenolic compound. The highest level of flavonoids (38.87 µg/mg) was detected in T. brownii bark, whereas

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racuity of Applied Biological Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan e-mail: mitunaga@gifu-u.ac.jp the highest amount of tannin was detected in *A. nilotica* (L.) bark (88.01 %).

**Keywords** Sudanese medicinal plants · DPPH · *Propionibacterium acnes* · Lipase inhibitor · Combretaceae

## Introduction

The unique and diverse flora of Sudan and its prominent traditional medicinal plants could probably be a rich source of bioactive compounds [1]. Information about Sudanese folk medicine was documented during comprehensive ethnobotanical investigations [2]. Acne vulgaris is a common skin disease of humans caused by bacteria inducing non-inflammatory and inflammatory skin lesions [3]. Propionibacterium acnes has been recognized as an obligate anaerobic organism which is usually found as a normal skin commensal. This organism has been implicated over other cutaneous microflora in contributing to the inflammatory response of acne. It acts as an immunostimulator which can produce a variety of enzymes and biologically active molecules involved in the development of inflammatory acnes. These products include lipases, proteases, hyaluronidases, and chemotactic factors [4]. Current treatment for acne is mostly based on antibiotics such as clindamycin and tetracycline derivatives. However, antibiotic resistance for P. acnes is widely spread and is a critical problem worldwide [5]. During this decade, they have been extensively studied for new compounds from natural substances possessing antibacterial activity against acne-inducing bacteria [6].

Reactive oxygen species (ROS) are subsequently generated from the hyper-colonization of *P. acnes* and from ultraviolet exposure [7]. Therefore, substances that inhibit the growth of skin microorganisms and have anti-oxidant activity are of great interest, since they may cure or prevent various diseases related to the effects of free radicals and may be useful in the treatment of adult pimples.

In the present study, the first anti-acne screening of Sudanese medicinal plants is shown by antibacterial activity against *Propionibacterium acnes*, lipase inhibition and antioxidant activity.

## Materials and methods

#### Reagents

Dimethyl sulfoxide (DMSO), GAM Broth Modified "Nissui", glucose, (+)-catechin, yeast extract (Difco, France), nutrient broth (Difco, France), Folin Ciocalteu, bovine serum albumin (BAS) and Tween-80 (MP Biomedicals LLC, France) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Tetracycline hydrochloride was obtained from Sigma Chemical Co. Ltd (China). 2-(*N*-Morpholino)ethanesulfonic acid (MES) and gallic acid were from Nakalai Tesque, Inc. (Kyoto, Japan). Isopropyl methylphenol (IPMP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and vanillin were from TCI (Tokyo, Japan). Lipase kits were from Pharma Biomedical (Osaka, Japan).

#### Plant materials

The plants were collected in Khartoum and Gadarif states of Sudan in March 2011. Voucher specimens are deposited in the Horticultural Laboratory, Department of Horticulture, Faculty of Agriculture, University of Khartoum. A list of voucher numbers of the investigated species is shown in Table 1.

## Preparation of plant extracts

Plant materials were shade dried at room temperature and powdered before being extracted with methanol and 50 % (v/v) ethanol in water (ratio of 1 g sample:10 ml solvent) for 12 h three times. The extracts were filtered and the solvent was removed under vacuum using rotary evaporator. The concentrated extracts were then dried under freeze drying. The crude extracts in yields are listed in Table 1.

## Test microorganism

*Propionibacterium acnes* ATCC 6919 was obtained from Biological Resource Center (NBRC), National Institute of Technology and Evolution, Chiba, Japan. The bacterial colonies were maintained in a medium consisting of GAM broth modified "Nissui" 0.5 %, glucose 1.0 %, yeast extract 0.3 %, nutrient broth 0.5 %, and 0.2 % Tween-80.

Evaluation of antibacterial activity

The broth dilution method was used to determine the minimum inhibitory concentration (MIC) of each plant extract. This assay was determined as described by Chomnawang et al. [8]. Briefly, 100  $\mu$ l of each extract was serially diluted twofold with 10 % DMSO; 95  $\mu$ l of sterilized medium and inoculum 5  $\mu$ l were added to each well of a 96-well plate. The inoculum was prepared at the density of 1  $\times$  10<sup>6</sup> CFU/ml approximately. The broth culture was incubated for 72 h under anaerobic conditions. Extract concentrations at which there was no visually detectable bacterial growth were described as the MIC. Tetracycline hydrochloride and IPMP were used as a positive control. Each experiment was carried out in triplicate.

DPPH radical scavenging assay

This assay was performed as described previously by Batubara et al. [9]. The free radical scavenging activity of the extract was evaluated by decolorization of DPPH at 510 nm.

Preparation of crude lipase from *Propionibacterium* acnes

Lipases were produced under the same culture conditions. The cell suspension was centrifuged at  $900 \times g$  for 10 min at 4 °C. The precipitate was diluted in phosphate buffer saline (PBS) at pH 6.98. The bacteria in this solution were destroyed by micro homogenizing system (TOMY Micro Smash MS-100) at 4000 rpm for 30 s and centrifuged at  $5000 \times g$  for 60 s. The filtrate was collected and placed in a dialysis tube for 6 days. In a freeze drier, the crude enzyme was lyophilized to a powder form [9].

Lipase inhibitory activity assay

Lipase inhibitory activity assay determined by BABLB-DTNB method [10] was carried out using a commercially available kit (Lipase kits). Tetracycline hydrochloride and IPMP were used as the positive controls.

Analysis of phenolics

### Determination of total phenolic

The total phenolic assay was performed as described previously by Ainsworth and Gillespie [11]. Data are expressed as milligram of gallic acid equivalents.

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Table 1	Anti-acne	properties	of	Sudanese	medicinal	plant ex	xtracts
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No.	Scientific name	Family	Used part	Voucher specimen	Extract	Yield (%) <sup>a</sup>	MIC (mg/ml)	Lipase inhibition (%) <sup>c</sup>	Antioxidant IC <sub>50</sub> (µg/ml) <sup>c</sup>
1	Abrus precatorius L.	Fabaceae	Seeds	SD-OD-22	М	16.8	_b	$51.59 \pm 0.92$	$7.97\pm0.29$
2					Е	6.80	2.00	$76.79\pm0.80$	$8.631 \pm 0.05$
3	Ambrosia maritima L.	Asteraceae	Aerial parts	SD-SH-03	М	16.3	2.00	$36.9\pm0.03$	$30.79\pm0.40$
4					Е	4.18	4.00	$28.48\pm0.11$	$40.56\pm0.60$
5	Citrullus coloynthis (L.) Schrad	Cucurbitaceae	Dry Fruit	SD-OD-05	М	34.5	4.00	$1.11\pm0.45$	nd
6					Е	12.0	4.00	$6.91\pm0.15$	nd
7	Ziziphus spina-christi (L.) Desf.	Rhamnaceae	Fruits	SD-SH-06	Μ	48.8	-	nc	nd
8					Е	6.89	4.00	$2.21\pm0.52$	$33.13 \pm 2.27$
9			Bark		М	36.8	2.00	$53.15\pm0.44$	$3.62\pm0.56$
10					Е	5.54	-	$35.40 \pm 1.21$	$6.22\pm0.70$
11			Leaves		Μ	47.1	2.00	$14.94\pm0.24$	$24.57\pm0.44$
12					Е	9.23	-	nc	$21.88\pm0.57$
13	Lawsonia inermis L.	Lythraceae	Leaves	SD-SH-07	М	39.6	1.00	$22.20\pm0.09$	$11.87 \pm 1.37$
14					Е	6.20	4.00	$11.62 \pm 0.19$	$15.36\pm1.20$
15	Moringa oleifera Lam.	Moringaceae	Leaves	SD-SH-08	М	20.3	-	$18.08\pm0.06$	$50.25 \pm 4.57$
16					Е	12.0	-	$27.34\pm0.07$	$18.47\pm0.52$
17	Salvadora persica L.	Salvadoraceae	Leaves	SD-SH-09	М	36.5	1.00	$3.16\pm0.18$	nd
18					Е	5.76	2.00	$12.03\pm0.18$	$24.50\pm5.62$
19			Stems		М	18.4	-	$5.83\pm0.95$	$71.61\pm0.63$
20					Е	5.17	4.00	$1.47 \pm 1.25$	nd
21	Tamarix nilotica (Ehrenb.) Bunge	Tamaricaceae	Stems	SD-OD-10	М	40.5	4.00	$34.49 \pm 0.30$	$10.92 \pm 0.63$
22					Е	12.2	-	$3.84\pm0.19$	$8.30\pm0.43$
23	Solenostemma argel Hayne		Leaves	SD-SH-23	М	37.2	-	$6.34\pm0.05$	nd
24					Е	13.5	-	$16.31\pm0.18$	$24.37\pm3.41$
25	Xanthium brasilicum W.	Compositae	Leaves	SD-SH-12	М	23.4	4.00	$16.73\pm0.08$	$24.71\pm4.44$
26					Е	9.7	-	$8.27 \pm 0.04$	$24.38\pm4.25$
27	Khaya senegalensis (Desr.) A. Juss.	Meliaceae	Bark	SD-SH-14	М	62.1	-	$20.12\pm0.76$	3.37 ± 0.68
28					Е	5.75	4.00	$38.73\pm0.28$	$12.20\pm1.17$
29	Balanites aegyptiaca (L.) Del.	Balanitaceae	Leaves	SD-SH-15	М	25.5	-	$12.28\pm0.29$	nd
30					Е	19.0	-	nc	nd
31			Fruit		М	74.4	4.00	nc	nd
32					Е	6.45	4.00	$6.91\pm0.07$	nd
33			Wood		М	5.64	4.00	$25.06\pm0.47$	nd
34					Е	3.15	2.00	$2.39\pm0.10$	nd
35	Hibiscus sabdariffa L.	Malvaceae	Flowers	SD-SH-48	М	58.4	4.00	$3.64\pm0.04$	$22.14\pm0.64$
36					Е	27.48	4.00	$16.15\pm0.19$	$68.13 \pm 4.25$
37	Abutilon pannosum (Forst.f.) Schlecht.		Leaves	SD-SH-43	Μ	16.1	2.00	2.43 ± 0.23	nd
38					Е	9.45	4.00	$17.46\pm0.05$	92.098
39	Terminalia brownii Fres.	Combretaceae	Bark	SD-GF-02	М	43.0	0.50	$51.67\pm3.62$	$3.85\pm0.37$
40					Е	4.46	2.00	$59.91 \pm 1.76$	$5.89\pm0.09$
41			Wood		М	12.5	4.00	$15.53\pm0.98$	$4.97\pm1.69$
42					Е	1.85	4.00	$54.30\pm0.26$	$5.19\pm0.93$

### Table 1 continued

No.	Scientific name	Family	Used part	Voucher specimen	Extract	Yield (%) <sup>a</sup>	MIC (mg/ml)	Lipase inhibition (%) <sup>c</sup>	Antioxidant $IC_{50}$ (µg/ml) <sup>c</sup>
43	Combretum hartmannianum (Schweinf.)		Bark	SD-KH-04	М	21.9	1.00	$45.76\pm0.69$	$4.99\pm0.44$
44					Е	4.88	2.00	$48.34\pm0.79$	$6.7\pm0.33$
45			Wood		М	4.68	0.50	$10.19\pm0.14$	$9.74\pm0.67$
46					Е	1.86	0.50	$40.45\pm0.07$	$8.21\pm0.25$
47	<i>Terminalia laxiflora</i> Engl & Diels		Wood	SD-KH-03	М	14.3	0.13	$69.51\pm0.45$	$4.44 \pm 0.13$
48					Е	2.04	0.13	$74.14\pm0.17$	$3.45 \pm 1.33$
49	Guiera senegalensis J.F.Gmel		Leaves	SD-OD-40	М	26.2	_	$25.78\pm0.79$	$6.30\pm0.59$
50					Е	12.7	4.00	$42.27 \pm 1.50$	$10.93\pm0.42$
51	Acacia seyal var seyal Del.	Mimosaceae	Bark	SD-GF-05	М	32.9	2.00	$42.33\pm3.47$	$4.03\pm0.51$
52					Е	6.44	4.00	$33.23 \pm 1.92$	$6.96\pm0.66$
53			Wood		М	2.52	_	$3.01 \pm 1.13$	$8.87 \pm 0.93$
54					Е	2.17	_	$20.21 \pm 0.11$	$9.71 \pm 1.79$
55	Acacia seyal var fistula (Schweinf.)		Bark	SD-GF-06	М	27.1	-	$25.22 \pm 2.21$	$7.53\pm0.11$
56					Е	6.88	_	$1.31\pm0.09$	$10.28\pm1.23$
57			Wood		М	8.54	4.00	nc	nd
58					Е	2.74	_	$19.24\pm0.01$	nd
59	Acacia tortilis (Forssk.) Hayne		Bark	SD-KH-07	М	5.70	4.00	nc	$15.01\pm0.04$
60					Е	2.30	4.00	$7.75 \pm 1.36$	$27.39 \pm 1.82$
61			Wood		М	5.70	4.00	$1.41 \pm 2.63$	$9.87 \pm 0.73$
62					Е	6.53	4.00	$21.29\pm0.19$	nd
63	Acacia nilotica (L.)		Pods	SD-OD-01	М	70.5	4.00	$75.35\pm0.58$	$1.32\pm0.97$
64					Е	6.15	2.00	$53.09 \pm 1.12$	$8.11\pm0.64$
65			Bark		М	23.75	_	$66.53 \pm 0.97$	$3.34\pm0.33$
66					Е	5.42	1.00	$53.84\pm0.35$	$3.20\pm0.68$
67	<i>Kigelia africana</i> (Lam.) Benth.	Bignoniaceae	Fruits	SD-SH-21	М	26.2	_	nc	$24.99 \pm 1.25$
68					Е	8.65	1.00	$15.22\pm0.24$	$37.34 \pm 6.47$
69	Hyphaene thebaica (L.) Mart.	Palmae	Fruits	SD-OD-41	М	52.9	4.00	$0.67\pm0.02$	nd
70					Е	14.4	4.00	$7.95 \pm 1.39$	$18.42\pm0.20$
71	<i>Moringa oblongifolia</i> (Frosk.) A. Rich	Capparidaceae	Stems	SD-SH-45	М	10.7	4.00	nc	nd
72					Е	4.20	4.00	$6.46\pm0.17$	$20.79 \pm 2.30$
73	Capparis deciduas (Forsk.) Edgew.		Stems	SD-SH-17	М	15.9	-	nc	nd
74					Е	4.13	4.00	nc	nd
75	Polygonum glabrum Willd.	Polygonaceae	Leaves	SD-SH-A- 03	М	33.7	4.00	$30.79\pm0.55$	$6.88\pm0.16$
76					Е	8.18	4.00	$31.02\pm0.54$	$5.170 \pm 0.38$
77	Solanum dubium Fresen	Solanaceae	Fruits	SD-SH-34	М	37.2	_	$2.61\pm0.11$	$10.66\pm0.41$
78					Е	10.9	4.00	$7.36\pm0.10$	$13.77\pm2.53$
79	Grewia tenax (Forsk.)	Tiliaceae	Fruits	SD-OD-42	М	69.9	_	nc	nd

#### Table 1 continued

No.	Scientific name	Family	Used part	Voucher specimen	Extract	$\begin{array}{c} \text{Yield} \\ \left(\%\right)^a \end{array}$		Lipase inhibition (%) <sup>c</sup>	Antioxidant IC <sub>50</sub> (µg/ml) <sup>c</sup>
80					Е	8.46	-	$3.58\pm0.12$	183.6 ± 31.81
	IPMP						1.00	$48.28\pm0.41$	*
	Tetracycline hydrochloride <sup>d</sup>						0.13	$64.06\pm0.26$	*
	(+)-Catechin						*	*	$2.39\pm0.97$

MIC minimum inhibitory concentration, M methanol extract, E 50 % ethanol extract, nc inhibition could not be calculated because there is no activity at the highest concentration of 500 µg/ml, nd failed to achieve 50 % inhibition at highest concentration 166.7 µg/ml

<sup>a</sup> Based on dried weight

<sup>b</sup> No inhibitory activity at concentration of 4.00 mg/ml

 $^{\rm c}\,$  Data given as mean  $\pm$  standard deviation of triplicate test

<sup>d</sup> MIC value in µg/ml

\* Not tested

#### Vanillin assay for flavonoids

This assay was performed as described previously by Hagerman [12]. The flavonoids in the plant extracts were expressed as (+)-catechin equivalents.

## BSA assay for Tannin

Tannin content of plant extracts was determined by a protein precipitation method using BSA. This method was described by Batubara et al. [13] by analyzing the BSA content of the supernatant liquid. Samples were diluted in 50 % (v/v) ethanol/water to prepare 2 mg/ml of concentration. A 200 µl volume of sample solution was added to 200 µl of BSA solution (10 mg/ml, dissolved with 0.1 M acetate buffer, pH 5). After reaction at room temperature for 1 h, the solution was centrifuged at  $13000 \times g$  for 2 min. The remaining BSA in the supernatant was determined by HPLC with a reversed phase Develosil300C4-HG-5 column (4.6 i.d. ×150 mm, Nomura Chemical Co, Ltd, Japan) monitored at 280 nm. The solvent system used was as follows: a linear gradient elution for 20 min from 80 to 20 % solvent A (0.01 % TFA in water) in solvent B (90 % (v/v) CH<sub>3</sub>CN/water containing 0.01 % TFA) at flow rate 1 ml/min. The column temperature was 35 °C.

### **Results and discussion**

In continuation of our research group studies to obtain antiacne agents from plant origin, we deal in the present work with the screening of 80 extracts belonging to 29 plant species distributed among 20 families. Generally, polar organic solvents are most effective at producing extracts high in phenolic compounds and antioxidant activity [14]. In this study, we used methanol and 50 % ethanol as solvent for plant extraction. The anti-acne activity of some Sudanese medicinal plant extracts were determined using an antibacterial assay, lipase inhibitory activity and a DPPH radical scavenging assay. Table 1 summarizes the scientific name, family, part used and voucher specimen of the medicinal plants. Within these selected Sudanese medicinal plants, *Lawsonia inermis* L., *Combretum hartmannianum*, *Acacia seyal* var fistula and *Solanum dubium* are used for preventing the dryness and bacterial infection

of the skin; other plants are used for anti-inflammation, anti-malaria, anti-diarrhea, antimicrobial infection and other diseases [15]. The antibacterial MIC values of the extracts of 29 medicinal plants used are presented in Table 1. Gibbson [16] suggested that isolated phytochemicals should have MIC <1 mg/ml, so that in this study MIC value 0.13 mg/ ml was considered to be an indication of excellent anti-

ml was considered to be an indication of excellent antibacterial activity. Among the extracts used in this study, the species belong to Combretaceae family comparatively showed a good antibacterial activity, specifically all extracts from T. laxiflora showed the best antibacterial activity against P. acne (MIC 0.13 mg/ml), followed by methanol extract of T. brownii (bark) and all of C. hartmannianum (wood) extracts exhibited a medium (MIC 0.5 mg/ml) antibacterial activity. According to Elegami et al. [17], water and methanol extracts of leaves, fruit and stem bark from C. hartmannianum exhibited an activity against gram-positive bacteria which was due to flavonoids, saponins, terpenes and tannins. Antimicrobial investigation of some species of Terminalia and Combretum that belong to Combretaceae indicated that the most effective extracts were the methanol extracts of roots of T. sambesiaca, T. kaiserana, T. sericea, C. fragrans and C. padoides. All these extracts showed a remarkable growth inhibition against gram-positive bacteria [18]. Although the activity of T. arjuna (bark) against acne bacteria is known [19], potency of T. laxiflora (wood) against P. acnes has not yet been investigated.

Total phenolic Sample Flavonoids Total tannins  $(\mu gGAE/mg)^{a}$ (µgCAE/mg)<sup>b</sup> (% BSA/mg)<sup>c</sup> no. 2  $34.98 \pm 2.83$ nd nd 39  $47.89 \pm 4.99$  $38.87 \pm 1.20$  $82.83 \pm 10.38$ 45  $36.01 \pm 1.77$ nd  $28.70 \pm 20.54$ 46  $35.49 \pm 3.31$  $31.73 \pm 21.62$ nd  $0.14 \pm 0.01$  $41.09 \pm 3.74$ 47  $81.91 \pm 9.921$ 48  $36.72 \pm 1.63$ 73.31 ± 17.79 nd 63  $44.40 \pm 4.15$  $53.68 \pm 9.30$  $18.55 \pm 0.27$  $35.73 \pm 4.83$ 65  $26.17\pm0.55$  $88.01 \pm 9.42$ 

 Table 2
 Total
 phenolic,
 flavonoid
 and
 total
 tannin
 contents
 of
 selected
 plant
 extracts
 as anti-acne
 activities

nd not determined

<sup>a</sup> Values expressed as gallic acid equivalent (GAE) per mg of plant extract

<sup>b</sup> Values expressed as catechin equivalent (CAE) per mg of plant extract

<sup>c</sup> Values expressed as percentage of BSA precipitated per mg of plant extract

DPPH is a stable radical that is used as a popular method of screening for free radical scavenging ability, antioxidant activity in particular of plant extracts [20]. This assay was used to evaluate the free radical scavenging activity of our plant extracts. The result in Table 1 showed that ten plant extracts have partially good potent activity with <5 µg/ml of IC<sub>50</sub>, comparing with positive control of (+)-catechin (IC<sub>50</sub> 2.39 µg/ml). In addition, methanol extract of *Acacia nilotica* (pod) demonstrated the lowest IC<sub>50</sub> for scavenging activity of the DPPH radical (IC<sub>50</sub> 1.32 µg/ml) and this could be due to some isolated compounds from this plant, such as galloylated catechins and gallocatechin derivatives [21]. Furthermore, Singh et al. [22] have mentioned that the strong antioxidant activity of *A. nilotica* (bark and leaves) was due to umbelliferone.

Results of the lipase inhibitory activity shown in Table 1 clarified that the methanol extract of *A. nilotica* (pod), 50 % ethanol extracts of *Abrus precatorius* (seed) and *T. laxiflora* exhibited the strongest inhibitory effect on lipase with more than 70 % inhibition. These values were higher than those of the positive controls such as tetracycline hydrochloride and IPMP at the same concentration. Methanolic extract of *T. laxiflora* and *A. nilotica* bark showed the second strongest activity with an inhibition of 69.5 and 66.0 %, respectively. Seven plant extracts expressed moderately strong activity with more than 50 % inhibition. Batubara et al. [9] reported that some extract from Indonesian medicinal plants had better activities than the positive controls (chloramphenicol, tetracycline, and IPMP).

A study done by Falcocchio [23] indicated that (+)catechin and kaempferol are promising candidates for treatment of acne due to their strong inhibitory activity on *P. acnes* lipase GehA (glycerol-ester hydrolase A), as well as to their wide anti-acne properties and their low toxicity. Previously, phytochemical studies of *A. nilotica* resulted in the identification of a variety of phenolic constituents, among which kaempferol was identified [24]. The best extract based on comprehensive activities was *T. laxiflora* 50 % ethanol extract with better MIC value 0.13 mg/ml, antioxidant activity (IC<sub>50</sub> 3.45 µg/ml) and lipase inhibitory activity (74.1 %).

The chemical analysis was performed to find the perspective chemical group which was responsible for biological activity. Total phenolic and flavonoid contents of active extracts were determined spectrophotometerically, while total tannin content was determined by the ability of BSA precipitation, which refer to condensed and hydrolysable tannins presence in the extracts. Vanillin HCl method is widely used to estimate the proanthocyanidin in the extracts. The result for total phenolic, flavonoid and tannin contents of selected extracts are presented in Table 2. Almost all selected extracts contained phenolics ranging from 34.9 to 47.9 µg/mg. Total phenolics content previously reported had relationship with antioxidant activity [25]. Many useful properties of flavonoids have been reported including anti-inflammatory activity, enzyme inhibition and antimicrobial activity [26]. The highest amounts of flavonoids (38.87 µg/mg) were detected in methanol extract of T. brownii bark and the lowest amount  $(0.14 \ \mu g/mg)$  was detected in methanol extract of T. laxiflora wood.

Tannins exhibit many biological activities. In previous studies, tannins have been evaluated as antibacterial, antiviral, radical scavenging, antitumor activities and inhibitory activities for some enzymes [27, 28]. No tannin content was detected in *A. precatorius* fruit. Methanolic extracts of *A. nilotica* (L.) bark, *T. brownii* bark and wood extracts (methanol, 50 % ethanol) of *T. laxiflora* have partially high percentage of total tannin (88.01, 82.83, 81.91 and 73.3 %) respectively. Among them, the extracts of *T. laxiflora* wood have less or no flavonoids; these results indicate that hydrolysable tannins may included in these extracts, while condensed tannin would be included in *A. nilotica* (L.) and *T. brownii* barks.

In conclusion, these findings demonstrated that the study of medicinal plants from Sudan confirmed a promising inhibitory effect in anti-acne. Fractionation and bioassayguided isolation of the anti-acne active pure compounds would be made on the crude extract of *T. laxiflora*. We also aim in our future studies to perform the chemical characterization (structure elucidation) of some of the anti-acne potent compounds. Toxicity and pharmacological studies are also needed to support the safety of these plants for cosmetic uses.

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