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Research Article

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Phytochemical analysis of *Buchanania axillaris* leaves extract

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Abstract

The present study carried out on phytochemical analysis of *Buchanania axillaris* leaf extract. The extraction was done with sohlet extraction method using methanol. The methnol extract was analyzed for its phytochemical constituents using standard test procedures and depends on results; thin layer chromatography was studied using different mobile phases. Then the extract used for isolation of bioactive molecules using column chromatography. The results of the phytochemical analysis says that, methanolic extract of *B. axillaris* leaves posses different phytochemicals like steroids, phenolics, flavanoids etc., and on chromatographic isolation, three compounds were isolated (BAL-01, 02, 03). The isolated compounds were identified as beta-sitosterol, quercetin, kamepferol based on structural elucidation studies (IR, MP, and NMR). The isolated compounds are first time reporting from the *B. axillaris.*

Key words: *Buchanania axillaris*, Leaves and Phytoconstituents.

1. Introduction

Human society has been dependent for ages on different natural resources to meet its requirements of food, medicine, fuel, timber and other such needs. Several hundreds of plants and animals in our surroundings are effectively being used for these purposes since long ago (Diamond, 2002). Of these, the plants used for food and medicines are usually considered important and attract attention because of their life supporting and life saving virtues (Iris F. F. Benzie and Sissi Wachtel-Galor, 2011). Herbal medicines are popular as remedies and play a key role in the human health care of a vast majority of world's population. According to world health organization (WHO) as much as 4 billion people (80 %) of the world's population rely on the use of traditional medicine which is predominantly based on medicinal plant materials (Ekor, 2013). Usually, these medicinal plant products have biologically active compounds and act as a lead compound for new drug discovery process. This means the lead compound, can be produced by total synthesis, or can be a starting point (precursor) for a semi synthetic compound, or can act as a template for a structurally different total synthetic compound (Lahlou, 2013). The potential use of medicinal plants as a source of new drugs is still poorly explored. In most cases, only pharmacological screening or preliminary studies have been carried out on medicinal plants (Rates, 2001). As natural product research continues to be an important part of the drug discovery, we selected the one of unexplored medicinal plant, *Buchanania axillaris* belongs to the family Anacardiaceae. *B. axillaris* is a traditional medicinal plant, have been using in the hyperdipsia, burning sensation cough braonchitis, dyspepsia, leprosy and constipation (Madhavachetty *et al.*, 2008). Some researchers reported anti-inflammatory, Anticancer and cardioprotective activities of leaf extracts (Madhavachetty *et al.*, 2008; Dorababu *et al.*, 2013; Dorababu *et al.*, 2016). But, the phytochemical analysis on *B. axillaris* very less reported. So, the present work aimed on we selected the phytochemical analysis *B. axillaris* leaves extract.

2. Materials and Methods

2.1 Plant material collection and preparation of extracts

Buchanania axillaris Desr leaves were collected from of the Thalakona region, Chittoor district, India. The plant specimen was authenticated by Dr. K. Madhava chetty, Department of Botany, Sri Venkateswara University, Thirupati. The plant materials were shade dried, then powdered in mill and extracted separately with methanol using soxhlet extraction process.

2.2 Chemicals

All the chemicals and reagents used were of analytical grade. Silica gel 100-200 mesh purchased from Sigma Aldrich.

2.3 Phytochemical analysis

The extract of *B. axillaris* was separately tested for various chemical constituents like steroids, alkaloids, tannins, flavonoids, carbohydrates, glycosides, tannins, phenolics, amino acids and quinines using standard test procedures **(**Pulok K. Mukherjee, 2002)**.**

2.4 Isolation of compounds

After the qualitative analysis of phytichemical constituents, Thin Layer Chromatography (TLC) was carried and on examination of the TLC results, the methanolic extract was chromatographed on silica gel (Cloumn Chromatography) and successively eluted (each 200 ml fraction) with n-hexane, chloroform and methanol.

3. Results and Discussion

The methanolic extract of *B. axillaris* was analyzed for different phytochemical constituents. The extract gave positive sterols, triterpenoids, flavonoids, phenolics, glycosides and also alkaloids (Table 1). The methanolic extract of *B. axillaris* showed six clear spots on TLC. The methanolic extract of *B. axillaris* also displayed similar spots in (Chloroform: Benzene (9:1), 1 % methanol in chloroform) on TLC by spraying with 5% methanolic sulphuric acid followed by heating. Hence, methanolic extract were subjected and loaded in chromatographic column over silica gel (100-200 mesh size) and eluted with solvents like hexane, chloroform and methanol and their mixtures in order of polarity, the residue afforded three compounds which were designated as BAL-01, BAL -02, BAL -03 (Table 2).

Table 1. Phytochemical analysis of methanolic extract of *B. axillaris* leaves.

Phytochemical constituents	**Methanolic extract of *B. axillaris***
Steroids	+
Trterpenoids	+
Glycosides	+
Saponins	-
Flavonoids	+
Tannins	-
Carbohydrates	+
Alkaloids	+
Amino acids	-
Oils	-
Quinones	-
Phenols	+

Table 2. Column chromatography of *Buchanania axillaris* leaf extract.

Combination of Solvents	**Fractions**	**Compounds**
Hexane	0-16	Waxy substance
Chloroform: hexane (10:90)	16-24	Waxy substance
Chloroform: hexane (20:80)	25-35	Waxy substance
Chloroform: hexane (30:70)	36-46	**BAL -01**
Chloroform: hexane (40:60)	47-60	Intractable substance
Chloroform: hexane (50:50)	61-75	Intractable substance
Chloroform: hexane (60:40)	76-84	Intractable substance
Chloroform: hexane (70:30)	85-99	Intractable substance
Chloroform: hexane (80:20)	100-116	Intractable substance
Chloroform: hexane (90:10)	117-130	Intractable substance
Chloroform	131-140	Wax
Methanol: Chloroform (1:99)	141-155	**BAL -02**
Methanol: Chloroform (2:98)	156-166	Intractable substance
Methanol: Chloroform (3:97)	167-177	**BAL -03**

BAL-01 (50 mg) eluted in chloroform: hexane (30:70) (fractions 36-46) afforded obtained as white amorphous solid like substance in wax. Repeated crystallization from hexane, it afforded pure white amorphous solid of and was identified as β – Sitosterol.

On continuation of elution a solvent system of methanol: chloroform (1:99) (fractions 141-155) yielded a yellow crystalline waxy needle, further purified by preparative TLC (methanol:chloroform 1:9). Repeated crystallization from hexane it obtained pure yellow crystalline needles of BAL-02 (50 mg) and was identified as kaempferol.

BAL-03 (55 mg) eluted in methanol: chloroform (3:97) (fractions 167-177) as yellow crystalline solid in wax, further purified by preparative TLC (methanol: chloroform 1:9). Repeated crystallization from hexane it obtained pure crystalline solid of and was identified as quercetin.

The physical characteristics of the isolated compounds yielded from leaf extract of *B. axillaris* in Table 3.

Table 3. Physical characteristics of the isolated compounds.

Isolated compound	Appearance	Melting point	R_f value in TLC
BAL-01	White amorphous solid	125-127^{0}C	0.50 (acetone: toluene)
BAL-02	Yellow crystalline needles	279-280^{0}C	0.56 (methanol: chloroform)
BAL-03	Yellow crystalline solid	312-314^{0}C	0.47 (methanol: chloroform)

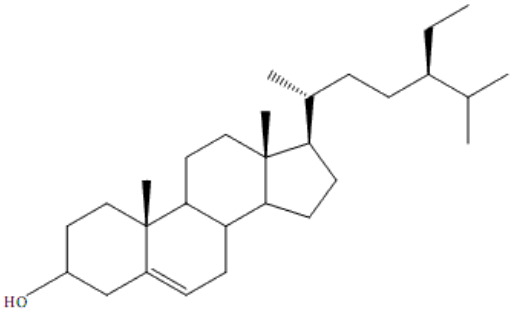

Fig 1. BAL-01 (β- Sitosterol)

BAL-01 (β – Sitosterol)

The fractions (36-46) were mixed as they were similar on TLC (chloroform: hexane 30:70) and recrystallized as needles from petroleum ether, mp is136-138^{0}C, $[\alpha]_D^{30}$ -36^0 (chloroform) and analyzed for the formula $C_{29}H_{50}O$. It showed color reactions in Libremann-Burchard test, characteristic of sterols (play of colours) and gave a single spot on silver nitrate impregnated TLC. Its IR showed peaks at 3440 (-OH), 1380 and 1385 cm^{-1} (gemdimethyl). It formed a monoacetate, m.p. 125-127 ^{o}C, $[\alpha]_D^{30}$ -38.2^0 (Chloroform). ^{1}H NMR spectrum showed the peaks at 0.80-1.25 (methyls), 4.45 (1H broad C-3H) and 5.30 (1H, m C-5H). From the above properties, compound BAL -01was identified as β-sitosterol (Fig 1) and the identity was confirmed by comparison with an authentic sample through melting point (mp) and Co-TLC.

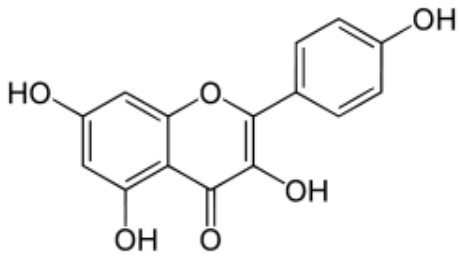

Fig 2. BAL-02 (Kaempferol)

BAL-02 (Kaempferol)

The fractions (141-155) were combined, as they were similar in Paper Chromatography (PC) and on recrystallization from chloroform, yellow crystalline needles, mp 279-280^{0}C and analyzed for the formula $C_{15}H_{10}O_6$ (fig 2). In UV light, it showed a single yellow spot and on exposing to ammonia, it turned to bright yellow. It gave positive color reactions characteristics for flavonols. An orange red precipitate with neutral lead acetate and a yellow color with Wilson's citric-boric acid reagent confirmed the presence of a free 3-hydroxyl and 5-hydroxyl groupings respectively. It formed a tetra acetate, mp 186-188 ^{0}C and tetra methyl ether, mp 163-164 ^{0}C. The UV spectrum in methanol had absorptions at λ_{max}^{MeOH} 253 s h, 265, 294 s h, 322 s h, 365 nm. Sodium acetate gave 10 nm bathochromic shift in Band II indicating the presence of a free 7-hydroxyl. With aluminum chloride / HCl, it formed a complex and showed a shift of 55 nm in Band I which further confirmed the presence of 3-OH group.

$NaOAc/H_3BO_3$ reagent did not give any pronounced shift, which suggested the absence of a free ortho-dihydroxy system. From the above properties, compound BAL-02 was identified as kaempferol and the identity was confirmed by comparison with an authentic sample through mp and co-TLC.

BAL-03 (Quercetin)

The fractions 167-177 were mixed, as they were similar in PC and the compound was crystallized from chloroform and methanol as yellow crystalline solid, mp 312-314^{0}C and analyzed for the formula $C_{15}H_{10}O_7$ (Fig 3). In PC, it was yellow under UV and intense yellow under UV/NH_3. With ferric chloride, it gave green color and with magnesium + HCl (Shinoda test), magenta color, characteristics for flavonoids. An orange red precipitate with neutral lead acetate confirmed the presence of 3-hydroxyl. Presence of 5-hydroxyl group was inferred through Wilson's boric and citric acid reaction. It formed penta acetate, m.p. 194-196^{0}C and a pentamethyl ether, mp. 150-151^0 C. UV showed absorption at $\lambda_{\max}^{MeOH}$ 257, 267sh, 301sh, 370 nm. A 55 nm Band I bathochromic shift with $AlCl_3$/HCl suggested the presence of 5-hydroxyl. Presence of B ring ortho-dihydroxyl grouping was indicated by a 15 nm Band II bathochromic shift with $NaOAc/H_3BO_3$ reagent. A bathocromic shift of 18 nm in Band II with NaOAc suggested the presence of 7-hydroxyl. ^{1}H-NMR exhibits peaks at 6.15 (d, 6H), 6.4 (d, 8H), 6.90 (d, 5'H), 7.60 (d, 6'H) and 7.75 (d, 2'H). The elemental analysis of the compound and its acetate and spectral data indicated that the compound is quercetin and the identity was confirmed by comparison with an authentic sample through m.p and co-PC.

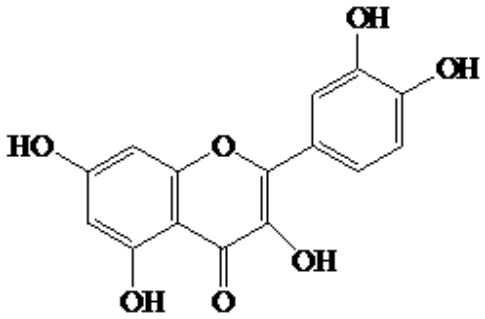

Fig 3. BAL-03 (Quercetin)

The results of present study, phytochemical analysis of *Buchanania axillaris* leaves extract provide the evidence that the medicinal plant produces different bioactive molecules. Those molecules play important role in their biological processes and are also helpful to humans in treating the diseases, if they are isolated in pure form or they serve as precursors for synthesis of leading bio active molecule.

The isolated molecules in the present study are bet-sitoterol, Quercetin and Kaempferol were previously reported from different medicinal plants and these molecules posses some biological activities (Soodabeh, 2014; Bushra Sultana and Farooq Anwar, 2008; Calderón-Montaño *et al.*, 2011), but now these compounds are first time reporting from selected plant species. But, further studies are needed on this plant for isolating different bioactive molecules using different solvents, because the elution or extraction of the phytoconstituents from medicinal plants depends on the polarity basis.

4. Conclusion

From the results of the present study it may conclude that the *Buchanania axillaris* possess different phytochemical compounds in it and they have different biological activity. Those compounds may act individually or synergistically in biological activities.

Acknowledgments

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Conflict of Interest

We have none to declare.

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