Antioxidants Activies of the Phenolic Constituents of Flowers and Leaves of Vernonia Elaegnifolia DC

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Abstract:

The essential phenolic compounds from the leaves and Inflorescence of V.elaeagnifolia was evaluated and resulted in the isolation and characterization of Kaempferol, P-coumaric acid and $Kaempferol - 3 - 0 - \beta - [6'' - E - P - coumaroyl] - D - glucopyranoside.$ *detailed UV visible and IR spectrometer as well as chromatographic technique data have been provided for the above compound.*

Keyword: *Vernonia elaeagnifolia*, Phenolic compounds: Kaempferol, P-Coumaric acidand $Kaempferol - 3 - 0 - \beta - [6'' - E - P - coumaroyl] - D - glucopyranoside.$

1. Introduction:

Vernoniae is one of the major tribes of the largest sunflower family of Asteraceae among 25000 species. These groups of plant occur as herbs, shrubs, or small trees in tropical America, Africa, Madagascar and Asia Vernoniae is recorded as medicine plants possessively effective against inflammation and ulceration of the mucous membrane of respiratory tract. It also a good source of dietary fiber, protein and minerals and thus serve as a rich source of bioactive constituents. The leaves and flowers of *V.elaeagnifolia* were investigated for poly phenolics and the resulted in the isolation of kaempferol, p-coumaric acid and $K - 3 - 0 - \beta - [6'' - E - P - coumaroyl] - D - glucopyramaside..$

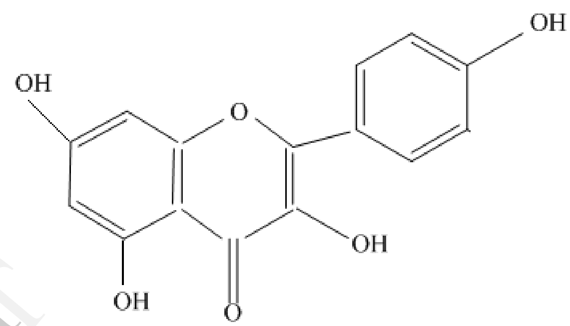


Fig.1. $\boldsymbol{Kaemferol}$

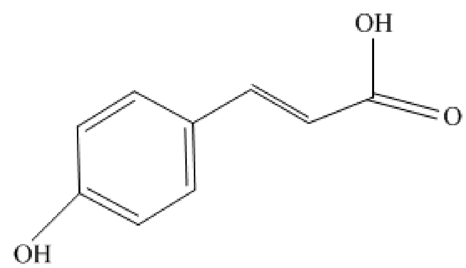
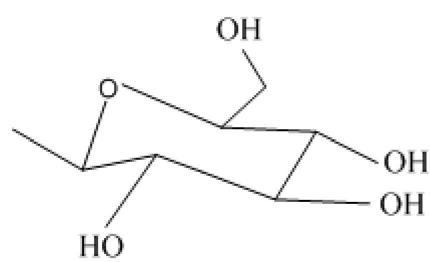


Fig.2. p – coumaric acid



β – D - glucopyranoside

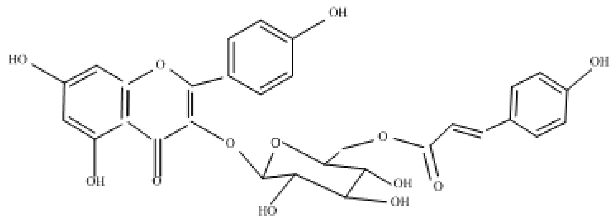


Fig.3. $\boldsymbol{K3-0-\beta-[6''-E-p-coumaryl]-D-glucopyranoside}$

2. Results and Discussion

From the alcoholic extract of the fresh healthy and uninfected aerial parts plant three flavonoid were isolated.

A systematic phytochemical analysis of the compounds resulted a flavonoid in the identification of a flavonoid aglycone Kaempferolglucoside,$K3-0-\beta-[6''-E-P-coumaroyl]-D-glucopyranoside$ and a phenolic acid paracoumaric acid.

2.1. Experimental

2.2. Kaempferol

Crystallized as yellow needles from Me_2CO, having the mp $277-279^{\circ}C$ and corresponded to the mol. Formula $C_{15}H_{10}O_6$. It produce pink colour with $Mg-Hcl$, yellow with alkalis and green with neutral ferric chloride and appeared yellow under UV and UV/NH_3, paper chromatographic mobilities corresponded to authentic Kaempferol.

2.3. UV(λ_{max}., nm)

MeOH : 254sh, 266, 302sh, 365

+NaOAc : 275, 301, 385(dec)

+NaOAc + H_3BO_3:267, 296sh, 320sh, 370

2.4. p – Coumaric acid

It crystalized from $MeOH-Me_2CO$(1:1) as colourless needles, mp,$203-204^{\circ}$, $C_9H_8O_3$. It produced pale yellow colour with alkalis, greenish brown with neutral ferric chloride and decolourised Br_2/H_2O. It was acidic to phenol red and produced effervescence with HCO_3^- Colourless under UV changing to bluish violet under UV/NH_3 and paper chromatographic mobilities corresponding to authentic p – coumaric acid.

UV(λ_{max}., nm)

MeOH : 255, 306

IR(v_{max}., cm^{-1})

KBr : 3390, 2750, 1660, 1625, 1595, 1510, 1235, 1205, and 830

2.5. Kaempferol $\boldsymbol{3-0-\beta-[6''-E-P-coumaroyl]-D-glucopyranoside}$

Pale yellow needles from MeOH, $C_{30}H_{26}O_{13}$, mp $262-264°$. It gave colour tests characteristic of flavonoid and answered Molisch's test. It was purple under UV, changing to yellow under UV/NH_3.

UV(λ_{max}., nm)

MeOH :254sh, 267sh, 298sh, 318, 360sh

+NaOAc :275, 298sh, 316, 372

+NaOAc + H_3BO_3:267, 299sh, 316sh, 359

+NaOMe :276, 310sh.371

+$AlCl_3$:276, 308, 320sh, 358, 395sh

+$AlCl_3$ + Hcl :276, 308,318sh, 356sh, 390sh

2.6. Acid hydrolysis of Compound C

(Kaempferol, p-coumaric acid, D-glucose)

Compound C, upon acid hydrolysis (2N HCl) an aglycone, identical in all aspects to compound A and a phenolic acid, resembling compound B in its behavior and hence were respectively identified as kaempferol and p-coumaric acid. The sugar was identified as D-glucose, by usual means.

2.7. Alkaline hydrolysis of Compound C

(Kaempferol 3-0-β-D-glucose, p-coumaric acid)

Compound C (20mg) in 2% NaOH solution (10 mL) was kept at room temp. Under N_2 atmosphere for 24h with constant stirring. The reaction mixture was acidified with dil. HCOOH and extracted with ether. The ether solution was washed, dried and concentrated. The residue was crystallised from Me_2CO to give colourless needles, mp$219 - 220°C$, identical in respects to compound B (p-coumaric acid) and thus got identified.

The aqueous layer was extracted with EtOAc and concentrated. The residue was recrystallized from MeOH when yellow needles mp$176 - 178°C$ was obtained. This fragment of the compound was indistinguishable, in colour reactions, UV fluorescence, UV absorption characteristics and Rf values in various solvent systems from authentic kaempferol 3 – 0 – β – D- glucoside confirming its identity.

Compounds	H_2O	5%	15%	50%	n-BAW	Phenol	Forestal	t-BAW
Kaempferol	00	00	05	42	93	68	62	89
P-Coumaric acid	83	46	56	82	90	81	87	94
$(K3 - 0 - \beta - [6'' - E - P - coumaroyl] - D - glucopyranoside)$	10	15	34	76	90	85	85	90
$K3 - 0 - \beta - D - glucopyranoside$	13	40	43	72	71	71	83	84

Table 1: R_f values of the phenolic compounds isolated from *V.elaeagnifolia*
(R_f x 100, Whatman No.1, ascending 28 ± 2˚C

n-BAW :n-BuOH:HOAc:H_2O, 4:1:5, Upper layer
Phenol :Water saturated phenol
t-BAW :t-BuOH:HOAc:H_2O, 3:1:1,

3. Materials and Methods

Fresh inflorescence of *Vernonia elaeagnifolia* (2.0 Kg) collected from Pondicherry, in the month of November were extracted with boiling 90% EtOH (4 x 12 L) under reflux and concentrated under reduced pressure. The crude aqueous concentrate was fractionated into C_6H_6, Et_2O and EtOAc (4 x 2 L each) solubles. The C_6H_6 extract that failed to indicate the presence of phenolic constituents was discarded and the other two fractions were stored in the ice-chest for a week. The C_6H_6 extracted that failed to answer the tests for phenols was discarded and the other two fractions were refrigerated for a week. The brownish mass Et_2O fraction, upon PC (Whatman No.3, 50% aq. HOAc, descending, 28˚, 10h) yielded yellow needles of compound A (hR_f = 42) and colourless crystals of compound B (hR_f = 82). The EtOAc fraction yielded another yellow solid, C.

3.1. Identification of Compound A(Kaempferol)

Compound A, $C_{15}H_{10}O_6$ mp $277 - 279°(Me_2CO)$ gave yellow colour with alkalis, pink with $Mg - HCl$ and olive green with Fe^{III}. It was yellow under UV and UV/NH_3 characteristic of flavonol with free 3-OH. it had λ_{max}. (MeOH) 254sh, 266, 302sh, 365nm. A bathochromic shift of 9nm in band II of NaOAc with decomposition of band I suggested free 3,7,4'-OH groups. NaOMe spectrum showing decomposition favoured the presence of free 3 and 4'-OH groups. A bathochromic shift of 55nm in band I of $AlCl_3/HCl$ spectrum suggested the presence of 3 and/or 5-OH groups. A hypsochromic shift of only 4nm in band I of $Alcl_3/Hcl$spectrum compared to $AlCl_3$ spectrum and no characteristics shift in band I of $NaOAc - H_3BO_3$ revealed the absence of orthodihydroxyl in ring-B. Based on the characteristic shift in the electronic absorption characteristic and coPC with authentic kaempferol[l21], the structure of compound A[1] was confirmed.

3.2. Identification of Compound B (p-Coumaric acid)

It got crystalized from$MeOH - Me_2CO(1:1)$ as colourless needles, mp,$203 - 204^{\circ}C C_9H_8O_3$. It produced pale yellow colour with alkalis, greenish brown with $Fe^{III}$and decolourisedBr_2/H_2O. It was colourless under UV and bluish violet under UV/$NH_3$and exhibited max. (MeOH)255, 306 nm. The IR absorption frequencies were observed at 3390, 2750, 1660, 1595, 1510 which were characteristic of unsaturated phenolic acid. Acetylation yielded monoacetate, mp 205-206$^{\circ}C$. These data led to the identification of compound B as p-hydroxycinnamic acid (p-Coumaric acid) (Fig 1).

3.3. Identification of Compound C

(Kaempferol $3 - 0 - \beta - [6'' - E - P -$ coumaroyl$] - D -$ glucopyramaside):

Compound C, pale yellow solid from MeOH,$C_{30}H_{26}O_{13}$, mp$262 - 264^{\circ}$, gave colour reactions characteristic of flavonoids and had λ_{max}. ($MeOH$) 254sh, 267, 298sh, 318, 360sh with the intensity of the band at 318 nm being strong. This indicated the presence of an additional chromophore like hydroxycinnamoyl moiety. Compound C, upon acid hydrolysis, yielded a flavonol, a phenolic acid and a sugar in approximately equal proportion. This flavonol aglycone had mp$277 - 279°C$ and UV absorption characteristics similar to compound A and based on its co-migration with authentic kaempferol, in solvents of varying polarities, the aglycone formed was identified as kaempferol. The phenolic acid was identical in all respects to compound B, including mmp with the authentic sample and hence was recognized as p-coumaric acid. The sugar component of the hydrolytic product had Rf in various solvent systems characteristic of D-glucose (Table 1)

Alkaline hydrolysis of compound C (2% NaOH) produced a flavonol glycoside and the same phenolic acid, viz., p-coumaric acid. The flavonol glycoside obtained, was crystallised as yellow needles from MeOH,$C_{21}H_{20}O_{11}$, mp$176 - 178°C$, became yellow in presence of alkalis, olive green with Fe^{III}, red with Mg-HCI and answered Molisch's test. It was purple under UV and yellow under UV/NH_3 and had max. (MeOH) 266, 298sh, 350nm, indicating the compound to be a 3-glycosylated flavonol. Otherwise the shifts in the UV absorption maxima were similar to compound A, suggesting it to be kaempferol 3-0-β-D-glycopyranoside [2] (fig.1). Acid hydrolysis of this glycoside provided an aglycone, indistinguishable from compound A and a sugar, D-glucose, identified by the usual procedure. Hence, the glycoside formed as a product of alkaline hydrolysis of compound C was confirmed, after co-PC and mmp with an authentic sample as K3-0-β-D-glucoside (astragalin). Thus, compound C could be confirmed as kaempferol 3-0-β-(6''E-p-coumaroyl)-D-glucopyranoside [3] (Fig.1).

4. Determination of Radical Scavenging Activity

4.1. Trolex equivalent Antioxidant capacity assay

This method relies on the generation of long-lived species 2, 2'-azinobis(3-ethylenebenzothiazoline-6-sulphonate)[$ABTS^{\cdot +}$] radical cationicchromophore in phosphate-buffered saline pH 7.4 and its quenching by an antioxidant, which is measured spectrophotometrically at 734nm. It determines the relative ability of the phenolic compounds to scavenge $ABTS^{\cdot +}$ as compared to a standard amount of Tolox (6-hydroxy-2,5,7,8-teramethylchroman-2carboxylic acid), the water-soluble vitamin E analogue, expressed as TEAC values. TEAC value is defined as the concentration of standard Trolox with the same antioxidant activity as 1mM concentration of the extract under investigation and is taken as an index to evaluate antioxidative activity of an antioxidant Authentic kaempferol[121] was used as a standard.

4.2. Auto oxidation of β - carotene

Oxidation of linoleic was measured by the method described by Pratt. Linoelic acid (20 mg) and Tween 20 (200 mg) were taken in a flask and a solution of β – carotene(2mg in 10ml of chloroform) was added. After removal of chloroform 50ml of distilled water saturated with oxygen was added. Aliquots (200 µl) of the ethenolic solution (µg/ml) of each compound were added to each flask with shaking. Samples without test extracts were used as blanks, and a sample with 2,6-di-tert-butyl-4-methylphenol (BHT) was used as a control substance. After incubation of the samples at 50˚C for 3 h to effect autoxidation, the absorbance was read at 470nm at regular intervals. The antioxidant activity as calculated using the relation, antioxidant activity = $\left[1-\frac{A_0-A_t}{A_{00}-A_{0t}}\right]\times 100$ where A_0= absorbance at the beginning of the incubation with test compound; A_t =absorbance at time t, with test compound; A_{00}= absorbance at the beginning of the incubation without test compound; A_{0t} = absorbance at time t, without test extracts. Compounds are considered active when their antioxidant activity is close to that of the control substance, BHT.

Conclusion

Compound 1.Kaempferol, compound 2.p-Coumaric acid and Compound 3.$K3-0-\beta-[6''-E-P-coumaroyl-D-glucopyranoside$, isolated from the fresh aerial parts of *V.elaegnifolia* have been found to exhibit, respectively, 1.75, 3.02 and 1.12 times potent antioxidant activity than either vitamins E or C, as evaluated by the Trolox equivalent antioxidant(TEAC) assay.

The antioxidative effect of the three compounds, on the antoxidation of linoleic acid was also measured employing bleaching of β-carotene as model system. The three compounds, isolated exhibited, respectively, about 30%, 78% and 34% of activities of the commercial antioxidant, $2{,}6-Di-tert-butyl-4-methylphenol$ (Butylated hydroxytoluene, standard control substance), BHT, when determined at 60 min., and about 25%, 66% and 29% at 120 min.

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