



## ESTIMATION OF POLYPHENOL CONTENT IN THE AQUEOUS EXTRACTS OF SOME PLANTS USED AS HERBAL DRUGS, STUDY THEIR ANTIOXIDANT ACTIVITY AND THERAPEUTIC EFFECTS

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### ABSTRACT:

Medicinal plants are frequently used as raw materials for extraction of active ingredients, which can be used in the synthesis of different drugs. This work incorporates the quantitative analysis of total poly-phenolics in plant extracts determined with the Folin-Ciocalteu reagent. Standard used for the analysis was gallic acid. Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. The standard graph was obtained for  $Y=0.0061x + 0.0396$ ;  $R^2=0.9991$ . Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. The antioxidant activity of the plant samples was determined using the DPPH radical-scavenging activity and FRAPS values. This study shows that the experiment was carried out through phytochemical analysis for the estimation of polyphenol content in the aqueous extracts of some plants used as herbals drugs and their antioxidant activity was studied. Elemental analysis confirmed the presence of sulfur, while nitrogen and halogens were found to be absent. Preliminary phytochemical screening of the extract indicated the presence of tannins, reducing sugars, **polyphenols, flavonoids, glycosides** etc.



**KEYWORDS:** Polyphenol, Herbal Drugs, DPPH, FRAPS, antioxidants, Folin-Ciocalteu.

### 1.INTRODUCTION

#### 1.1 General Concern

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, foodstuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. For the large proportions of world's

population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use. The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing

nations [AG, Atanasov, B Waltenberger, et.al, (2015)]. The foundations of typical traditional systems of medicine for thousands of years that have been in existence have formed from plants. The plants remain to offer mankind with new medicines. Some of the beneficial properties ascribed to plants have recognised to be flawed and

medicinal plant treatment is based on the experimental findings of hundreds to thousands of years. Therapeutic plants are much of the time utilized as crude materials for extraction of dynamic fixings, which can be utilized in the blend of various medications. These therapeutic plants consider as a rich assets of fixings, which can be utilized in medication improvement and combination. Other than that, these plants assume a basic job in the improvement of human societies around the entire world. Additionally, a few plants consider as significant wellspring of nourishment and therefore, these plants suggested for their remedial qualities. Like if there should be an occurrence of intestinal medicines, blood thinners, anti-infection agents and against jungle fever meds, contain fixings from plants. [RB Bassey, AA Osinubi et al. (2012)]. These days the expression "Elective Medicine" turned out to be regular as it center around utilizing the plants for therapeutic reason however the present conviction that medications which come in cases or pills are the main meds that we can trust and utilize. All things being equal, the greater part of these pills and cases we take and use during our day by day life originated from plants. In addition, the dynamic elements of Taxol, vincristine, and morphine are disengaged from foxglove, periwinkle, yew, and opium poppy, separately. These plants incorporate ginger, green tea, pecans and some others plants. Different plants and their subsidiaries are additionally considered as significant hotspot for dynamic fixings, which are utilized in headache medicine and toothpaste.

Most of medicinal plants have specific compounds, other than antioxidants, which are effective in the treatment or prevention of diseases. In this regard, medicinal plants have also been a reliable source for preparation of new drugs. Nowadays, researchers more than before are dependent on medicinal plants for discovery of new drugs with fewer side effects. Therefore, due to the importance of oxidative stress in the pathophysiology of most of the hard curable diseases, the use of medicinal plants with antioxidant properties is important and should be considered more than before. Drug therapy and even drug discovery should also be focused more than before on this source. Multidisciplinary team work including ethnobotanists, pharmacologists, physicians and phytochemists is essential for the fruitful outcome of medicinal plants research. More importantly, regulations are also needed to ensure efficacy, quality and safety of herbal medicines.

## 1.2 Polyphenols

**Polyphenols** also known as **polyhydroxyphenols** are a structural class of mainly natural, but also synthetic or semisynthetic organic chemicals characterized by the presence of large multiples of phenol structural units. The number and characteristics of these phenol structures underlie the unique physical, chemical, and biological (metabolic, toxic, therapeutic, etc.) properties of particular members of the class. Examples include tannic acid and ellagitannin. The historically important chemical class of tannins is a subset of the polyphenols. The earliest widely accepted definition of polyphenols, the White-Bate-Smith Swain-Haslam (WBSSH) definition, was offered and justified by natural product and organic chemist Edwin Haslam and co-workers, based on the earlier natural products research of Edgar Charles Bate-Smith, Anthony Swain, and Theodore White that characterized specific structural characteristics common to plant phenolics used in tanning (i.e., the tannins).

**1.2.1 Chemistry of Polyphenols:** The Polyphenol class can be described as follows:

- generally moderately water-soluble compounds
- with molecular weight of 500–4000 Da
- with >12 phenolic hydroxyl groups
- with 5–7 aromatic rings per 1000 Da

where the limits to these ranges are somewhat flexible.[AG.Atanasov, B, Waltenberger et-al (2015)][Saini N.K. and M. Singhal et-al (2012) ] The definition further states that polyphenols display unique physical and chemical behaviors related to their high molecular weights and profusion of phenolic substructures-precipitation of proteins and particular amine-containing organics (e.g., particular alkaloid natural products), and formation of particular metal complexes (e.g., intense blue-black iron(III) complexes). HPLC is a sensitive and accurate tool that widely used for the quality

assessment of plant extract and its derived product/formulation. RP-HPLC with C18 columns is the most popular technique for the analysis of polyphenols of the different food.

### 1.3 Herbal Plants under study:



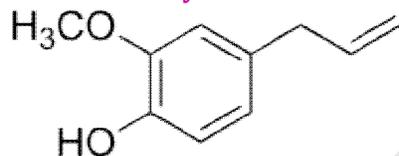
(a) *Nigella sativa* (Kalonji) (b) *Curcuma Longa* (Turmeric/Haldi) (c) *S. aromaticum* (Loung/Clove)

Fig. 1.1 The Plants Under Study used for herbal drugs

#### 1.3.1 The Appearance:

**CLOVE:** The clove plant is an evergreen herb that grows up to 8–12 m tall, with large leaves and crimson flowers grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green and then transition to a bright red when ready for harvesting. Cloves are harvested at 1.5–2.0 cm long, and consist of a long calyx that terminates in four spreading sepals, and four unopened petals that form a small central ball.

#### 1.3.2 Chemistry of Cloves:



The compound eugenol is responsible for most of the characteristic aroma of cloves. Eugenol composes 72–90% of the essential oil extracted from cloves and is the compound most responsible for clove aroma. 100% extraction occurs at 80 minutes in pressurized water of 125 °C. Ultrasound-assisted and microwave-assisted extraction methods provide more rapid extraction rates with lower energy costs. Other important essential oil constituents of clove oil include acetyl eugenol, beta caryophyllene and vanillin, crategolic acid, tannins such as bicornin, gallotannic acid, methyl salicylate (painkiller), the flavonoids eugenin, kaempferol, rhamnetin, and eugenitin, triterpenoids such as oleanolic acid, stigmasterol, and campesterol and several sesquiterpenes. Eugenol is toxic in relatively small quantities; for example, a dose of 5–10 ml has been reported as being a near fatal dose for a two-year-old child.

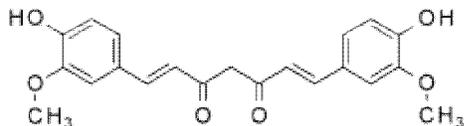
#### 1.3.3 The Appearance:

**TURMERIC:** Turmeric is an enduring herbaceous plant that scopes up to 1 m (3 ft 3 in) tall. Exceptionally spread, yellow to orange, round and hollow, fragrant rhizomes are found. The leaves are exchange and orchestrated in two lines. They are separated into leaf sheath, petiole, and leaf cutting edge. From the leaf sheaths, a bogus stem is framed. The petiole is 50 to 115 cm (20–45 in) long. The straightforward leaf edges are generally 76 to 115 cm (30–45 in) long and once in a while up to 230 cm (91 in). They have a width of 38 to 45 cm (15 to 18 in) and are oval to curved, narrowing at the tip. Turmeric is a blossoming plant, *Curcuma longa* of the ginger family, Zingiberaceae, the underlying foundations of which are utilized in cooking. The plant is a lasting, rhizomatous, herbaceous plant local

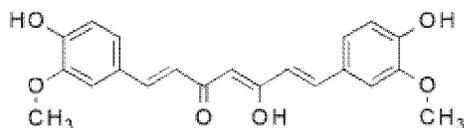
to the Indian subcontinent that requires temperatures somewhere in the range of 20 and 30 °C (68 and 86 °F) and a lot of yearly precipitation to flourish.

### 1.3.4. Chemistry of Turmeric:

#### Phytochemistry



#### Curcumin keto form



#### Curcumin enol form

Turmeric powder is around 60–70% starches, 6–13% water, 6–8% protein, 5–10% fat, 3–7% dietary minerals, 3–7% basic oils, 2–7% dietary fiber, and 1–6% curcuminoids. Phytochemical segments of turmeric incorporate diarylheptanoids, a class including various curcuminoids, for example, curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin establishes up to 3.14% of measured business tests of turmeric powder (the normal was 1.51%); curry powder contains substantially less (a normal of 0.29%). Some 34 basic oils are available in turmeric, among which turmerone, germacrone, atlantone, and zingiberene are significant constituents.

### 1.3.5. The Appearance:

**KALONJI:** *N. sativa* grows to 20–30 cm (7.9–11.8 in) tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually colored pale blue and white, with five to ten petals. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds which are used as spice, sometimes as a replacement for black cumin.

## 2. THE ANALYSIS:

The work was carried out in several phases as mentioned below.

- Selection of the herbal medicinal plants based on literature survey.
- Estimation of the polyphenol content in selected medicinal plants as, (Kalonji (*N. Sativa*), Haldi/Turmeric(*C.Longa*), Loung/Clove(*S.aromaticum*)).
- Estimation of the polyphenol content in selected plants.
- Screening of the aqueous extracts for its antioxidant activity.

## 3. MATERIALS AND METHODS

The phytochemical analysis techniques are those of extraction, isolation, structural elucidation, then quantification.

### 3.1 Chemicals and Reagents used:

Gallic acid, catechin, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), and 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), L-ascorbic acid, tannic acid, Folin-Ciocalteu's phenol reagents, and ferrous sulphate-heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) were purchased from Sigma Aldrich/Matshri Chemicals/Bengal Chemicals, India. All of the chemicals and reagents used in this study were of analytical grade.

### 3.2 Sample collection

Different varieties of Haldi, Loung, kalonji (*Curcuma Longa*, *Syzygium aromaticum*, *N.Sativa*) were collected from the **Sarguja district of Batouli division of Chhattisgarh** in India. Collected plant

samples were packed into sterile polybags before transportation to the Laboratory of Biochemistry and Phyto-Chemistry Department of Dr. C.V. Raman University Bilaspur (C.G.)

### 3.3 Phytochemical analysis

#### 3.3.1 Plant Extract Preparation of Stems and fruits, and Yield Determination:

Plants samples were cleaned and air-dried in the shade for two days before being ground to a fine powder in a blender (Systronics, Mumbai, India). The fine powder was used to prepare both ethanolic and aqueous extracts based on Kang's methods [Naseri M. et-al (2004)] with slight modification. Briefly, 20% ethanolic extract was prepared by adding grinded plant powder (20g) in 70% ethanol solution to make a 100 ml solution. Similarly, for aqueous extract preparation, 20 g of the powder was dissolved in water to make a 100ml solution. Both ethanol and aqueous extract solutions were placed in the dark to avoid reaction that may occur in the presence of light and were scan in the shaker for 72h at room temperature. Then the solution were filtered through whatman no. 1 filter paper and concentrated in a rotary evaporator under reduced pressure of 100psi at 40 °C (for ethanol) and 55 °C for water. The dried extracts were collected at 20° subsequent analysis the percentage of yield of the extract was determined according to the the following formula viz. % yield equals to the weight of sample extract / initial weight of smaple x 100. Further, eight different extracts were prepared for antioxidant analysis.

#### 3.3.2 Plant Extract preparation of Leaves and Tissues:

In this study we used two methods of obtaining the methanolic plant extracts. The first methanolic plant extract was prepared according to the method of Danesi et-al. 2014 with some modification. This method uses the minimum volume of reagents and almost eliminates wasted reagent. Briefly, 40 mg of crushed dried leaves were extracted with 1ml of 80% aqueous methanol and homogenized. The homogenates were incubated for 20 minutes at 70°C, in a Thermoblock TB2 and then centrifuged. The supernatants were collected and stored at -20°C until analysis. The second extraction was carried out as described by Muresan et al., 2012. For samples extraction, 1g of powdered materials were extracted with 10ml of methanol. The extract was separated and the tissue was re-extracted until the extraction solvents became colorless (the total solvent volume was between 100-200ml). The filtrates were combined in a total extract which was dried by vacuum rotary evaporator at 40°C. The dry residues were recovered in 7 ml of methanol and stored in a freezer at -20°C until analyzed.

### 3.4 Qualitative analysis of Total Phenolic content for all herbal plant samples

Quantitative analysis of total phenolics in extracts was determined with the Folin- Ciocalteu reagent. Standard used for the analysis were gallic acid. Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. The standard graph was obtained for  $Y=0.0061x + 0.0396$ ;  $R^2=0.9991$ . Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered and allowed to stand for 30 min at room temperature before the absorbance was at read at 760 nm spectrometrically. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Accordingly, total phenolic content can be determined. All determination was performed in triplicate for all three extracts. The total phenolic content (TPC) was measured using experimental measurements Crude extracts of plants were dissolved by methanol to obtain stock extract solution at concentration 500 µg/ mL (w/v). As standard antioxidants, BHA (2(3)-t-Butyl-4- hydroxyanisole) and BHT (3,5-di-tert-butyl-4 hydroxytoluene), were dissolved in methanol in concentrations of 100 µg/mL and tested for antioxidant activity. All of applied spectrophotometric measurements were performed using Systronics UV-VIS Double beam Spectrophotometer. Absorbance was recorded at 740 nm after two hours incubation. The same procedure was repeated for standard gallic acid in order to construct calibration curve. Phenolic content of samples was calculated from standard curve equation and expressed as gallic

acid equivalents (mg GAE/g dry extract). The total polyphenol content present was determined as gallic acid equivalent (GAE) (6.25, 12.50, 25.00, 50.00, 100.00  $\mu\text{g}/\text{mL}$ =0.9970) and was expressed as g of GAE/100g of herbal plant. The DPPH scavenging activity assay was performed according to a method reported by Odriozola-Serrano et al., 2008. A volume of 3.9 ml of methanolic DPPH solution was allowed to react in darkness, for 30 minutes with 10  $\mu\text{L}$  of samples and 90 $\mu\text{L}$  of  $\text{H}_2\text{O}$ . The absorbance was measured at 515 nm against methanol. The antioxidant activity was calculated as follows:

$$\% \text{Radical scavenging activity (RSA)} = (A_0 - A_1 / A_0) 100,$$

Where  $A_0$  was the absorbance of DPPH solution and  $A_1$  the absorbance of the sample. The analyses were run in triplicate and the results are expressed as average values with the standard error mean (SEM).

### 3.5 Antioxidant Activity

The antioxidant activity of the plant samples was determined using the DPPH radical-scavenging activity and FRAPS values.

#### 3.5.1 DPPH Free Radical-Scavenging Activity

The antioxidant activities of all plant extracts were evaluated according to the DPPH radical-scavenging activity as described by Braca et al. Briefly, 1 mL of the extract was mixed with 1.2 mL of 0.003% DPPH in methanol at varying concentrations (2.5-80.00  $\mu\text{g}/\text{mL}$ ). The percentage of DPPH inhibition was calculated using the following equation: where this is the absorbance of DPPH in the absence of a sample and is the absorbance of DPPH in the presence of either a sample or the standard. Absorbance of DPPH in the presence of either a sample or the standard. DPPH scavenging activity is expressed as the concentration of a sample required to decrease DPPH absorbance by 50% ( $\text{IC}_{50}$ ). This value can be graphically determined by plotting the absorbance (the percentage of inhibition of DPPH radicals) against the log concentration of DPPH and determining the slope of the nonlinear regression.

#### 3.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

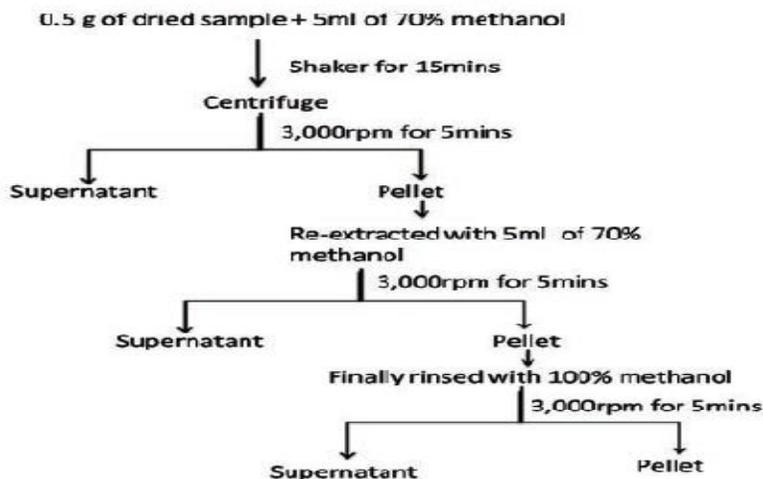
The FRAP assay was performed as described by Benzie and Strain. The reduction of a ferric tri-pyridyltriazine complex into its ferrous form produces an intense blue color at low pH that can be monitored by measuring the absorbance at 593nm. Briefly, 200 $\mu\text{L}$  of the extract solution at different concentrations (62.5-1000.0  $\mu\text{g}/\text{mL}$ ) was mixed with 1.5 mL of the FRAP reagent, and the reaction mixture was incubated at 37 $^{\circ}\text{C}$  for 4 Min. the FRAP reagent was prepared by mixing 10 volumes of 300M acetate buffer (pH 3.6) with 1 volume of 10 mM TPTZ solution in 40 Mm hydrochloric acid and 1 volume of 20 mM ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ). the FRAP reagent was prewarmed to 37 $^{\circ}\text{C}$  and was always freshly prepared. A standard curve was plotted using an aqueous solution of ferrous sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) (100-1000  $\mu\text{mol}$ ), with FRAP values expressed as micromoles of ferrous equivalent ( $\mu\text{M}$  Fe per 100 g of sample).

### 3.6 Statistical Analysis

All analysis was performed in triplicate, and the data are reported as the mean standard deviation (SD). Data were analyzed using SPSS (Statistical Packages for Social Science, version 16.0, IBM Corporation) and Microsoft Excel 2007). Statistical analysis of the biochemical data were conducted using Tukey's test. This was considered statistically significant.

## MATERIALS AND METHODS

### Extraction of Phenolic Compounds



These supernatants were pooled together before removal of methanol and the concentrated extract was dried and stored for further use.

## 4. RESULTS AND DISCUSSION

Polyphenols are a group of secondary metabolites involved in the hydrogen peroxide scavenging in plant cells. Interest in plant materials rich in polyphenolic compounds increase recently, due to their high antioxidant potency which may offer protection against chronic disease, such as cardiovascular diseases, neuronal disease, cataract and several forms of cancer.

### 4.1 Effect of Drying on Polyphenol Extractability

Polyphenol content of various tissues of herbal plants has been reported previously Naseri M. et.al (2004), but there is no report on the effect of drying treatment on polyphenol content of various tissues, especially the unripe fruit. There are several data that demonstrate the effects of drying treatment on the polyphenol content and the efficiency of warm water (50-60°C) as a solvent for the extraction of polyphenolic compounds from different tissues. It was evident from the data that the applied drying processes did not affect the polyphenol contents and the extractability of polyphenols by water. However, microwave dried sample gives slightly higher yield of polyphenol compared to oven- and air-dried samples. Similar results were reported by Chan et al. (Bassam Abdul Rasool Hassan et-al (2012). For leaf extract. Lin et al (Khosravi-Boroujeni H, Mohammadifardrt-al (2012) reported similar results for green tea. Results of the colorimetric analysis of total phenolics are given here and are based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solution of gallic acid equivalents. The free radical scavenging activities of the obtained extracts from four salvia species are given also in table.

**Table 1. Studied Effect of Drying on Polyphenol content and water extraction efficiency of different tissue of herbal plants.**

Drying Treatment	Tissue	Extraction	TPC (mg GAE/100g)	Yield (100%)
Microwave	Leaves	1 <sup>st</sup>	264.93 ± 15.46	86.74 ± 4.43
		2 <sup>nd</sup>	36.43 ± 0.18	11.72 ± 0.06
		3 <sup>rd</sup>	9.63 ± 0.21	3.10 ± 0.07
	Stems	1 <sup>st</sup>	252.19 ± 2.17	89.14 ± 0.77
		2 <sup>nd</sup>	22.36 ± 0.20	7.90 ± 0.07
		3 <sup>rd</sup>	8.37 ± 0.38	2.96 ± 0.14
	Fruits	1 <sup>st</sup>	325.19 ± 1.54	86.80 ± 0.41
		2 <sup>nd</sup>	38.71 ± 1.12	10.33 ± 0.30
		3 <sup>rd</sup>	10.74 ± 0.59	2.87 ± 0.16
Air	Leaves	1 <sup>st</sup>	228.00 ± 4.12	87.44 ± 1.58
		2 <sup>nd</sup>	24.80 ± 0.18	9.51 ± 0.07
		3 <sup>rd</sup>	7.95 ± 0.15	3.05 ± 0.06
	Stems	1 <sup>st</sup>	213.05 ± 2.07	88.35 ± 0.86
		2 <sup>nd</sup>	20.52 ± 0.28	8.51 ± 0.12
		3 <sup>rd</sup>	7.56 ± 0.09	3.31 ± 0.04
	Fruits	1 <sup>st</sup>	319.57 ± 0.87	90.26 ± 0.25
		2 <sup>nd</sup>	25.88 ± 0.21	7.31 ± 0.06
		3 <sup>rd</sup>	8.59 ± 0.04	2.43 ± 0.01
Oven	Leaves	1 <sup>st</sup>	252.52 ± 2.21	86.86 ± 0.76
		2 <sup>nd</sup>	29.76 ± 0.06	10.23 ± 0.02
		3 <sup>rd</sup>	8.46 ± 0.19	2.91 ± 0.06
	Stems	1 <sup>st</sup>	216.24 ± 6.38	88.06 ± 2.60
		2 <sup>nd</sup>	21.49 ± 0.43	8.75 ± 0.17
		3 <sup>rd</sup>	7.84 ± 0.08	3.19 ± 0.03
	Fruits	1 <sup>st</sup>	323.76 ± 3.75	88.63 ± 1.03
		2 <sup>nd</sup>	32.21 ± 0.36	8.82 ± 0.10
		3 <sup>rd</sup>	9.32 ± 0.15	2.55 ± 0.04

Values of total phenolic content (TPC) are means ±SD(n=3)

**Table 2 The content of total poly-phenols of Kalounji, Haldi, Loung, (N.Sativa, Curcuma Longa, SyzygiumAromaticum,) samples**

Plant Species	Total phenols (mg GAE/g)		DPPH%	
	Methods 1	Methods 2	Methods 1	Methods 2
<b>(a) Nigella sativa (Kalonji)</b>	3672.16±0.142	2725.04±0.962	93.37±0.72	80.14±1.09
<b>(b)Curcuma Longa(Turmeric/Haldi)</b>	3220.11±0.050	2489.84±0.832	90.16±51	78.56±0.98
<b>(c)S. aromaticum(Loung/Clove)</b>	2890.34±0.069	1983.78±0.092	87.38±0.85	64.95±0.42

## 5. CONCLUSION

The results of this study show that, investigation was made and experiment was carried out through phytochemical analysis for the estimation of polyphenol content in the aqueous extracts of some plants used as herbals drugs and their antioxidant activity was studied. Detailed chemical investigations were undertaken to analyse the basic chemical nature, to establish uniformity in quality standard of the plants under research for herbal drugs and to identify the specific polyphenolic compounds in them. Elemental analysis confirmed the presence of sulfur, while nitrogen and halogens were found to be absent. Preliminary phytochemical screening of the extract indicated the presence of tannins, reducing sugars, **polyphenols**, **flavonoids**, glycosides and salicylates. Among these, flavonoids are well known for their ability to inhibit pain perception and to exhibit antiinflammatory properties

due to their inhibitory effects on enzymes involved in production of the chemical mediator of inflammation. Flavonoids and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing the fever. Since flavonoids exhibit several biological effects such as antiinflammatory, antimicrobial, antihepatotoxic and antiulcer activities, it is likely that the antioxidant activity is primarily related to the presence of flavonoids. Therefore, standard hydrolysis procedure was followed for detailed investigation of its chemical constituents, especially polyphenols, flavonoids and glycosides, by using UV-Visible spectroscopy, TLC.

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