

RRST-Biochemistry

## Gallic Acid in Ayurvedic Herbs and Formulations

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Article Info	Abstract
<b>Article History</b> <i>Received</i> : 18-01-2011 <i>Revised</i> : 28-04-2011 <i>Accepted</i> : 29-04-2011	Thirty Ayurvedic herbs and formulations have been screened for the presence of gallic acid by the use of silica gel thin layer chromatography. The samples showing presence of gallic acid in screening by the silica gel thin layer chromatography were quantitatively analyzed for gallic acid by ferric reducing antioxidant power (FRAP) assay. The water extracts of nine samples out of thirty were found to contain gallic acid by TLC. All these samples contain a good amount of gallic acid with Amla 27.36mg/g ranking first followed by Triphala 18.24mg/g. The results demonstrated rich sources of gallic acid in some ayurvedic herbs and formulations, which might provide new light on uses of this medicinally important compound.
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©ScholarJournals, SSR	<b>Key Words:</b> Gallic acid, Ayurvedic herbs and formulations, TLC, FRAP assay

### Introduction

A large number of plants are rich sources of gallic acid either in free form or as a part of tannin molecule. It is present in tea, red wine, fruits, beverages and various medicinal plants (1-6).

Gallic acid is known to have anti-inflammatory, antimutagenic, anticancer and antioxidant activity (7-13). It also seems to have antifungal, antiviral (14) and antibacterial properties (15). Gallic acid was found to show cytotoxicity against cancer cells without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal haemorrhage (14). It has been found very beneficial in uterine, pulmonary, and nephritic haemorrhages. It has given benefit in purpura (16). It is used to treat albuminuria and diabetes (14). It is a known matrix-metalloproteinase inhibitor (17). All these properties make gallic acid a pharmacologically important compound.

Different ayurvedic herbs and formulations are already in use in treatments of different diseases over years. This present work deals with these herbs and formulations concerning with determination of gallic acid by thin layer chromatography. A rapid visualization is achieved by using Ferric chloride/Potassium ferricyanide reagent. The samples showing presence of gallic acid in screening were quantitatively analyzed for gallic acid by ferric reducing antioxidant power assay, and the amount of gallic acid in these samples was estimated.

### Materials and Methods

#### Chemicals

Gallic acid was purchased from HiMedia Laboratories Pvt. Limited. 2, 4, 6-tripyridyl- 1, 3, 5-triazine (TPTZ) was purchased from Sigma Aldrich. Silica gel 60 F<sub>254</sub> plates were

purchased from Merck. All other chemicals used in the study were of AR grade.

#### Ayurvedic Samples

Thirty Ayurvedic herbs and formulations (as shown in Table1) were purchased from market.

#### Sample preparation

The powders of all herbs and formulations were defatted with hexane, and then suspended in water (1:6) and kept overnight. Next day all the extracts were centrifuged at 10×1000 rpm for five minutes and the supernatants were used for further chromatographic analysis.

#### Standard preparation

For standard preparation 1 in 1000 solution of gallic acid was used.

#### Thin layer chromatographic technique

This technique used was as previously described (18). About 10µl of the sample and 10µl of the standard were applied on silica gel plates. Thin layer chromatograms were developed by using a mixture of 5 volumes of chloroform, 4 volumes of ethyl formate and 1 volume of formic acid as a solvent system. The development was stopped when the solvent front had advanced about 7.5 cm. After drying plates in air, for sometime, a mixture of 1 volume of 1 in 100, 10% ethanol solution of ferric chloride and 1 volume of 1 in 100, 50% ethanol solution of potassium ferricyanide was used as a spraying agent for the detection. Gallic acid in the sample was identified by comparison with the spot of the reference standard.

**Quantification of gallic acid**

**Sample preparation**

The samples showing presence of the gallic acid in screening by the thin layer chromatographic technique were again applied on the silica gel plates. Thin layer chromatograms were developed by using the solvent system described above. The portions of chromatograms containing gallic acid were scrapped and suspended in a known volume of water and kept overnight. Next day, extracts were centrifuged at 10x1000 rpm for five minutes and supernatants were quantitatively analyzed for the gallic acid by ferric reducing antioxidant power assay (FRAP Assay).

**FRAP working solution**

25 ml of acetate buffer (300 mM, pH 3.6), 2.5 ml of 2, 4, 6 tripyridyl -1, 3, 5-triazine (TPTZ) (10mM) in 40 mM HCl and 2.5 ml of ferric chloride hexahydrate (FeCl<sub>3</sub> 6H<sub>2</sub>O) (20mM). The reagent was prepared freshly before use.

**FRAP assay**

Ferric reducing antioxidant power assay was assessed according to Benzie and Strain (19) using spectrophotometer. The method is based on the reduction of Fe<sup>3+</sup>-TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 593 nm. Briefly 3 ml of working FRAP reagent prepared daily was mixed with the adequate amount of the diluted sample; the absorbance at 593

nm was recorded after a 30-minute incubation at 37°C. FRAP values were obtained by comparing the absorption change in the samples with those obtained from the standard.

Gallic acid was used as a standard (100 µM-1000 µM) and the data were based on the experiment carried out in duplicate.

**Results and Discussion**

Thin layer chromatographic analysis has shown that nine samples contain gallic acid (Figure 1). Further quantitative analysis of these samples was done by ferric reducing antioxidant power assay. These samples and formulations contained 0.009-2.74% gallic acid with the Amla 2.74% ranking first followed by Triphala 1.82% and Khair 0.009% ranking lowest.

Table 2 shows the nine ayurvedic herbs and the formulations with the amount of gallic acid they contain.

These ayurvedic herbs and formulations are widely used in the treatment of different diseases; some of their properties that co-relate with the gallic acid can be discussed as shown in Table 3.

The pharmacological effects of these herbs and formulations might be due to gallic acid or it might be one of the contributors to these effects, however what contributory role the gallic acid play to these effects needs to be studied.

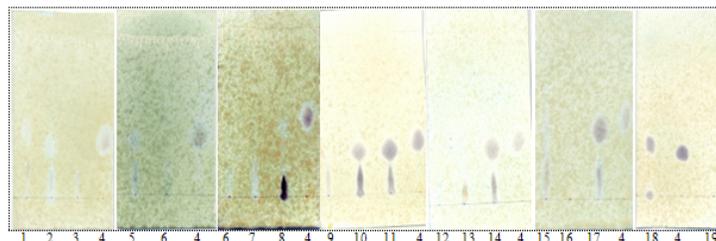


Figure 1. Silica gel thin layer chromatography of ayurvedic herbs and formulations with standard gallic acid. Solvent system used- chloroform - ethyl formate - formic acid (5: 4: 1). The numbers used in the figure indicate ayurvedic herbs and formulations : (1) *Acacia arabica* ( Babool), (2) *Acacia catechu* (Khair), (3)

*Liquorice glycerriza* (Jyeshthamadh), (4) Standard gallic acid, (5) *Eugenia jambolana* (Jamun), (6) *Nardostachys jatamansi* (Jatamansi), (7) *Symplocos racemosa* (Lodhra), (8) *Punica granatum* (Dalimb), (9) *Picrorhiza kurroda* (Kutki), (10) *Terminalia chebula* (Hirda), (11) *Terminalia bellerica* (Behda), (12) *Allium sativum* (Lasuna), (13) *Azadirachta indica* (Neem), (14) *Phyllanthus emblica* (Amla), (15) *Mentha arvensis* (Pudina), (16) *Boswellia serrata* (Shallaki), (17) Triphala, (18) Chyavanprash, (19) Spirulina (Tablets). The thin layer chromatograms of the *Alpinia galanga* (Kulinjan), *Piper longum* ( Pippali), *Cyperus scariosus* (Nagarmotha), *Hibiscus rosa-sinensis* (Jaswand), *Aloe indica* (Aloes), *Tylophora asthmatica* (Anantmul), *Pterocarpus santalinus* (Raktachandan), *Carica papaya* (Papaya), *Mimusops elengi* (Bakul), *Adhatoda vasica* (Adulsa), *Convolvulus plurivalis* (Shankhapusphi), *Rubia cordifolia* (Manjishtha) are not shown in figure, since the presence of the gallic acid has not been detected in these herbs.

**Conclusions**

The TLC analysis and the FRAP assay identified and quantified the gallic acid in the nine ayurvedic herbs and formulations. The results demonstrated that these herbs and formulations contain a good amount of the gallic acid. In addition

they suggest possible novel sources of the gallic acid, which might find pharmacological use. However, whether each of these herb and formulation administered in traditional dosages is sufficient to produce desired pharmacological effects needs to be investigated.

Table 1. Ayurvedic herbs and formulations

Sr.No.	Sample Name	Scientific Name
1.	Jyeshthamadh	<i>Liquorice glycerriza</i>
2.	Babool	<i>Acacia arabica</i>
3.	Khair	<i>Acacia catechu</i>
4.	Kulinjan	<i>Alpinia galanga</i>
5.	Pippali	<i>Piper longum</i>
6.	Nagarmotha	<i>Cyperus scariosus</i>
7.	Jaswand	<i>Hibiscus rosa-sinensis</i>

8.	Aloes	<i>Aloe indica</i>
9.	Anantmul	<i>Tylophora asthmatica</i>
10.	Jamun	<i>Eugenia jambolana</i>
11.	Raktachandan	<i>Pterocarpus santalinus</i>
12.	Jatamansi	<i>Nardostachys jatamansi</i>
13.	Pappaya	<i>Carica papaya</i>
14.	Bakul	<i>Mimusops elengi</i>
15.	Adulsa	<i>Adhatoda vasica</i>
16.	Kutaki	<i>Picrorhiza kurroda</i>
17.	Hirda	<i>Terminalia chebula</i>
18.	Behda	<i>Terminalia belerica</i>
19.	Lodhra	<i>Symplocos racemosa</i>
20.	Dalimb	<i>Punica granatum</i>
21.	Pudina	<i>Mentha arvensis</i>
22.	Shankhapushpi	<i>Convolvulus pluricalis</i>
23.	Manjishtha	<i>Rubia cordifolia</i>
24.	Shallaki	<i>Boswellia serrata</i>
25.	Lasuna	<i>Allium sativum</i>
26.	Neem	<i>Azadirachta indica</i>
27.	Amla	<i>Phyllanthus emblica</i>
28.	Triphala	---
29.	Chyvanprash	---
30.	<i>Spirulina</i> (Tablets)	---

Table 2. Gallic acid content of ayurvedic herbs and formulations

Sr.No.	Sample Name	Gallic acid in Mg Per gm the sample	Percent (%) gallic acid
1.	<i>Acacia Arabica</i> (Babool)	0.593 mg	0.059%
2.	<i>Acacia catechu</i> (Khair)	0.091 mg	0.009%
3.	<i>Eugenia jambolana</i> (Jamun)	1.094 mg	0.109%
4.	<i>Terminalia chebula</i> (Hirda)	7.144 mg	0.714%
5.	<i>Terminalia belerica</i> (Behda)	6.46 mg	0.646%
6.	<i>Punica granatum</i> (Dalimb)	1.915 mg	0.191%
7.	<i>Phyllanthus emblica</i> (Amla)	27.36 mg	2.376%
8.	Triphala	18.24 mg	1.824%
9.	Chyavanprash	2.234 mg	0.223%

Table 3. Medicinal uses of ayurvedic herbs and formulations

Sr.No.	Sample Name	Medical Use	References
1.	<i>Acacia Arabica</i> (Babool)	Diabetes (bark), Haemorrhagic diseases, Styptic	20, 21
2.	<i>Acacia catechu</i> (Khair)	Antibacterial, Antifungal, Antiinflammatory, Antioxidant (wood extract), Astringent, Anticancer (bark)	20, 22
3.	<i>Eugenia jambolana</i> (Jamun)	Diabetes (fruit, powdered seed kernel and it's aqueous extract), Astringent (bark, root, seed, leaves), Antibacterial (leaves)	20, 23, 24, 25
4.	<i>Terminalia chebula</i> (Hirda)	Diabetes, Antibacterial, Antioxidant, antitumor, Considerable effect in inhibition of HIV virus (fruit), Astringent	26, 27
5.	<i>Terminalia belerica</i> (Behda)	Astringent, Antibacterial (fruit)	20, 28
6.	<i>Punica granatum</i> (Dalimb)	Antibacterial (fruit rind and root bark), Astringent	20
7.	<i>Phyllanthus emblica</i> (Amla)	Anticancer, Haemorrhage, Antibacterial, Antiviral (dried fruit), Astringent, Diabetes (fruit), Antioxidant	28, 29, 30
8.	Triphala	Antibacterial, Antiviral, Antifungal, Anti-inflammatory, Antioxidant, Antitumor	31
9.	Chyavanprash	Antibacterial, Antioxidant	32

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