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ARTICLE

Results of quantitative and qualitative analyses of traditional prescription "Lonlunsemberu-13"

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Abstract: *Traditional prescription Lonlunsemberu-13 has been widely used in traditional Mongolian medicine to treat gastro intestinal dyspepsia.*
The purpose of this study was to determine biologically active substances in the Traditional prescription Lonlunsemberu-13 as a primary research.
We screened for phenolic compounds, flavonoids, coumarins and alkaloids using a TLC method, and then we have determined those biologically active substances content by the UV/Vis spectrophotometric method. We identified the gallic acid, rutin, quercetin, apigenin, luteolin, isofraxidin and piperine in the traditional prescription. The result revealed that "Lonlunsemberu-13" consisted 4.38% ±1.9 of total phenolic compounds, 0.63% ±0.17 flavonoids, 2.45% ±0.28 of coumarins and 0.83% ±0.064 alkaloids.

Keywords: *Lonlunsemberu-13; thin layer chromatography; UV/Visible spectrophotometer; polyphenolics; flavonoid; coumarin; alkaloid;*

INTRODUCTION

Traditional medicine has always played an important role in Mongolia's medicinal science. It continues to be practiced widely, supplying health-care needs of a large portion of the population. Folk medicine, based on the experiences of nomadic people, has its own unique medical theory, techniques and medications. Some aspects of Mongolian folk medicine along with elements from other Asian systems, such as Tibetan medicine, Ayurveda and traditional Chinese medicine have been integrated into the Mongolian medical system.

Traditional medicine practices and knowledge, including the use of medicinal plants, have been passed down from one generation to another via oral traditions.

Today, throughout the world there is intensive activity related to traditional medicine, ranging from extensive research into different plant species to their therapeutic application.

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The strong interest in traditional medicine, especially in naturally occurred and herbal medicine, is growing across the world. In Mongolian flora, about 860 from 2860 plant species were defined as having medicinal properties. Drugs with different properties can be developed based on broad pharmacological activities of flavonoids, alkaloids, coumarins, sesquiterpenoids and other biologically active compounds contained in plants. Mongolian scientists have conducted primary and advanced phytochemical research by using some 150 medicinal plants and herbs, which have been used in traditional Mongolian medicine [4, 6].

As recorded in traditional medical sources, "Lonlunsemberu-13" has been widely used in traditional Mongolian medicine to treat dyspepsia-gastrointestinal disorders.

"Lonlunsemberu-13" is composed of 13 medicinal herbs, including *Amomum kravanh Pire* ex gagner., *Amomum tsao Ko Grevost et Lemaire.*, *Terminalia chebula* Retz., *Alpinia officinarum* Hance., *Carthamus tinctorus* L., *Piper longum* L., *Piper nigrum* L., *Myristica fragrans* Houtt., *Purica granatum* L., *Cinnamomum cassia* Pres., *Nigella glandulifera*., *Halite* and *Halit violace* [2, 4]. These medicinal herbs contain bio-active compounds, such as flavonoids, coumarins, phenolic compounds, alkaloids and essential oil [3, 5, 7, 8, 14].

MATERIALS AND METHODS

Standards and Chemicals:

Folin-Ciocalteu reagent from Sangon (China), gallic acid, rutin, isofraxedin and piperine were acquired from Sigma Aldrich (USA). All other solvents and chemicals were of analytical grade.

The study was carried out in the Institute of Traditional Medicine and Technology of Mongolia. The "Lonlunsemberu-13" prescription was produced in the Traditional Medicinal Drug Factory of the Institute of Traditional Medicine. Biologically active compounds of the "Lonlunsemberu-13" prescription were studied by using UV/Vis spectrophotometry and TLC methods.

TLC identification of biologically active substances

Sample preparation: 0.5 g of samples was extracted in 10 mL 70% ethanol for 24 h, filtered and the filtrate was used as a test solution.

Identification of total phenolic compounds: Standard gallic acid substance was dissolved in 70% ethanol to produce a solution containing 4 mg per ml as the reference solution. The method for TLC was applied, using silica gel 60 F_{254} (Merck, Germany) plates as the coating substance and a mixture of benzene: ethyl acetate: formic acid: acetone - 5:5:2:0.5 as the mobile phase. After the development and removal of the plate, it was dried in air. It was sprayed with a 2% iron trichloride in ethanol and heated at 100-105^{0}C [3, 9, 11, 13].

Identification of flavonoids: Standard rutin, quercetin, apigenin and luteolin substances were dissolved in 70% ethanol to produce a solution containing 4 mg per ml as the reference solution. The TLC method was applied using silica gel 60 F_{254} plates as the coating substance and a mixture of toluene: ethyl: acetate: acetic acid- 4.5:5:0.5 and ethylacetate: acetic acid: formic acid: water - 100:11:11:26 as the mobile phase. After the development and removal of the plate, it was dried in air, and sprayed with a 3% ammonium chloride in ethanol [9, 11,13].

Identification of coumarins: Standard isofraxedin substance was dissolved in 70% ethanol to produce a solution containing 4 mg per ml as the reference solution. The TLC method was applied using silica gel 60 F_{254} plates as the coating substance and a mixture of toluene: ethyl acetate: acetic acid - 4.5:5:0.5 as the mobile phase. After development and removal of the plate, it was dried in air and sprayed with a 10% potassium hydroxide in ethanol [9, 10,13].

Identification of alkaloids: Standard piperine substance was dissolved in 70% ethanol to produce a solution containing 4 mg

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per ml as the reference solution. TLC method was applied using plates as the coating substance and a mixture of toluene: acetone: methanol: ammonia - 49.5:41.5:8:5 as the mobile phase. After development and removal of the plate, it was dried in air and sprayed with a Dradendorff's reagent [10,11, 13].

Quantitative UV/Vis spectrophotometric determination

Sample preparation: 0.5 g drug was extracted in 50 mL 70% ethanol solvent. After the extraction, the sample was filtered and the filtrate was transferred to a 50 mL volumetric flask, and to which 70% ethanol was added to facilitate sample extract (A solution).

Determination of total phenolic compounds content: The total content of phenolic compounds was determined spectrophotometrically by using a Folin-Chiocalteu reagent, which resulted in a dark blue complex compound.

Solution A 5 mL was transferred to a 25 mL volumetric flask, and diluted with 70% ethanol to the volume (B solution).

0.5 mL of solution B was transferred to a 25 mL volumetric flask containing distilled water (10 mL) and Folin Ciocalteu reagent (1 mL diluted tenfold). The solution was diluted with 10.75% sodium carbonate (w/v). The absorbance of the blue solution was measured after 30 minutes at room temperature at 760 nm in UV-Visible spectrophotometer - 1601 (Shimadzu, Japan) [1,12]. Distilled water was used as a blank.

Preparation of standard solution for calibration curve of gallic acid

15 mg of gallic acid reference substance, with constant weight, is weighed precisely and put into 50 mL volumetric flask with a small amount of distilled water and then diluted to the volume. It was shaken well, put aside. The exact amount is taken from solution 1, 3, 5, 7 and 9 mL placed in 25 mL volumetric flasks, respectively, and diluted to the volume. The final concentrations were 12, 36, 60, 84 and 108 µg/mL of galllic acid. Then, a mixture was prepared with 0.5 mL of this each stock solution, 1mL Folin-Ciocalteu reagent, and 10 mL of water, and volumetrically diluted to 25 mL with 10.75% sodium carbonate (w/v). After 30 min, the absorbance was measured at 760 nm, using water as the compensation liquid and quartz cell (1 cm path length) in a UV/Vis spectrophotometer.

Determination of total flavonoid content: 3 mL of solution A was transferred to a 25 mL volumetric flask containing distilled water (6 mL) and 5% NANO2 (1 mL). After standing for 6 minutes, 10% AlCl3 solution (1mL) and 4% NaOH (10 mL) were added and then diluted with distilled water to the volume. The absorbance of the red colored solution was measured after 15 min at room temperature at 500 nm in UV/Vis spectrophotometer-1601 (Shimadzu, Japan) [1, 10]. 70% ethanol was used as a blank.

Preparation of standard solution for calibration curve of rutin

10 mg of rutin reference with constant weight is weighed precisely and put into 50 mL volumetric flask with a small amount of 70 % ethanol and then diluted to the volume. It was shaken well and put aside. The exact amount is taken from solution 0.5, 1, 2, 3, 4 and 5 mL placed in 25 mL volumetric flasks, respectively, and added with distilled water (3 mL) and 5% $NANO_2$ (1 mL). After standing for 6 minutes, it was combined with 10% AlCl3 solution (1mL), 4% NaOH (10 mL) and diluted with distilled water to the volume. The final concentrations were 4, 8, 16, 24, 32 and 40 µg/mL of rutin. After 15 min, the absorbance was measured at 500 nm, in a UV/Vis spectrophotometer. 70% ethanol was used as a blank.

Determination of total coumarin content: 1 mL of solution A was transferred to a 25 mL volumetric flask diluted with 70% ethanol to the volume. The absorbance solution was measured after 15 min at room temperature at 336 nm in UV/Vis spectrophotometer-1601 (Shimadzu, Japan) [8, 14]. 70% ethanol was used as a blank.

Preparation of standard solution for calibration curve of isofraxedin

2 mg of isofraxedin reference substance with a constant weight is weighed precisely and put into 10 mL volumetric flask with small amount of 70% ethanol and then diluted to the

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volume. It was shaken well and kept aside. The exact amount is taken from solution 1.5, 2, 2.5, and 3 mL is placed in 10 mL volumetric flasks respectively and diluted to the volume. The final concentrations were 30, 40, 50 and 60 µg/mL of isofraxedin. After 15 min, the absorbance was measured at 336 nm, in a UV/Vis spectrophotometer. 70% ethanol was used as a blank.

Determination of total alkaloid content: 1 mL solution A was transferred to a 25 mL volumetric flask diluted with 70% ethanol to the volume. The absorbance solution was measured after 15 min at room temperature at 343 nm in UV/Vis spectrophotometer-1601 (Shimadzu, Japan) [5, 7,12]. 70% ethanol was used as a blank.

Preparation of standard solution for calibration curve of alkaloid

1 mg piperine reference substance with constant weight is weighed precisely and put into 50 mL volumetric flask with small amount of 96% ethanol and then diluted to the volume. It was shaken well and kept aside. The exact amount is taken from solution 1, 2, 4, 6, 8 and 10 mL placed in 10 mL volumetric flasks respectively and diluted to the volume. The final concentrations were 2, 4, 8, 12, 16 and 20 µg/mL of piperine. After 15 min, the absorbance was measured at 343 nm, in a UV/Vis spectrophotometer. 96% ethanol was used as a blank [5].

RESULTS AND DISCUSSION

The results of qualitative analysis of "Lonlunsemberu-13" are shown in Table 1 and quantitative analyses summarized in Table 2.

TLC results - total phenolic compounds (gallic acid), flavonoids (rutin, quercetin, apigenin, luteolin), coumarins (isofraxedin) and alkaloids (piperine) were identified in "Lonlunsemberu-13".

Gallic acid was identified in the solvent system benzene-ethylacetate-formic acid-acetone (5:5:2:0.5). It was dark-blue in color, Rf=0.88, on TLC plate.

Rutin, apigenin, luteolin and quercetin aglycones of flavonoid was identified in the solvent system toluone-ethylacetate-acetic acid (4.5:5:0.5) and ethylacetate - acetic acid - formic acid - water (100:11:11:26), which showed yellow and yellow fluorescence color; Rf values were 0.6, 0.82, 0.9 and 0.78 respectively. Also, Isofraxedin and piperine were identified in the solvent system toluene – ethylacetate - acetic acid (4.5:5:0.5) and toluene – acetone – methanol - ammonia (49.5:41.5:8:5). It showed blue fluorescence and orange color, Rf values were 0.61 and 0.85 respectively (Table 1).

These biologically active substances were contained in the components of raw materials of traditional prescription "Lonlonsemberu-13".

The researchers determined that plants, such as *Terminalia chebula* Retz., *Purica granatum* L., *Carthamus tinctorus* L., *Amomum kravanh Pire* ex gagner., *Alpinia officinarum Hance*., *Cinnamomum cassia* Pres, *Piper longum* L. and *Piper nigrum* L contained gallic acid, ellagic acid, tannic acid, ethyl gallate, chebulic acid, chebulagic acid, corilagin, mannitol, ascorbic acid, coumarins, flavonoids as a polyphenolic compounds and piperine, piperidine, trimethoxy cinnamoyl-piperidine, piperlongumine as alkaloids [5, 12, 14].

As a result of quantitative analysis, we determined the content of biologically active substances in traditional prescription, such as phenolic compounds, flavonoids, coumarins, and alkaloids, by using UV/Vis spectrophotometric method respectively.

The calibration curve of standard gallic acid, rutin, isofraxedin for the estimation of total phenolic, total flavonoids and total coumarins has been shown in Fig 2, 3, 4. While calibration curve of standard piperine for the quantification of total alkaloids has been shown Figure 5.

As shown in Figure 2, the result indicated gallic acid had linear relationship with peak area in the concentration range 12-108 µg/mL, calibration equation y = 110.77 x - 0.0736 (r^2= 09917). The RSD of gallic acid peak area was 4.2% (n=5).

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Table 1. Identification of bio-active compounds in sample

	Solvent system	Identification reagent	Color, after detection	R_f value
1	benzene-ethylacetate-formic acid-acetone (5:5:2:0.5)	2% $FeCl_3$ Heating (100^0C) Visible light	Dark-blue	0.88-Gallic acid
2	Toluone-ethylacetate-acetic acid (4.5:5:0.5)	3% $AlCI_3$ UV_{365} light	Yellow	0.6 - Rutin 0.82-Apigenin 0.9 –Luteolin
3	Ethylacetate- acetic acid- formic acid-water (100:11:11:26)		Yellow fluorescence	0.78-Quercetin
4	Toluone- ethylacetate-acetic acid (4.5:5:0.5)	10% KOH UV_{365} light	Blue fluorescence	0.61-Isofraxedin
5	Toluone- acetone-methanol-ammonia (49.5:41.5:8:5)	Dragendorff's reagent Visible light	Orange	0.85-Piperine

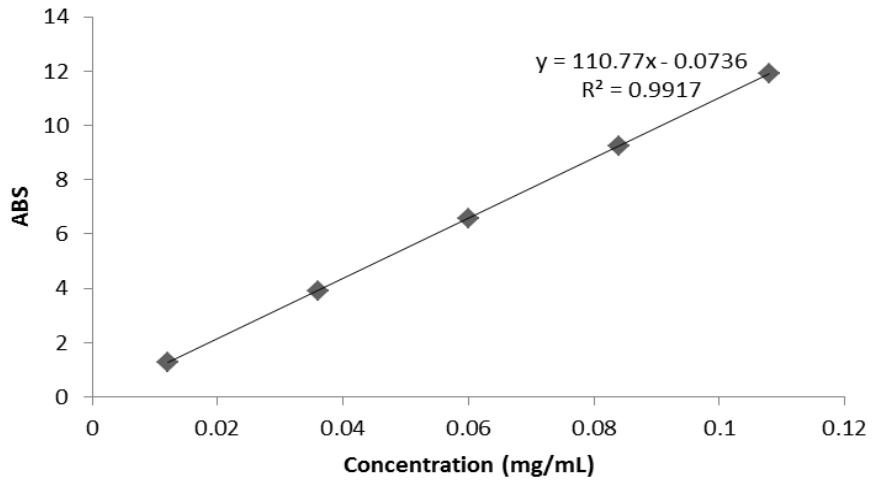

Figure 1. Gallic acid calibration curve

A good linear relationship was observed between absorbance and concentration of rutin in the concentration range 4-40 µg/mL, regression value of r^2= 09999, calibration equation y = 0.0955 x - 0.0008. The RSD was 0.16% (n=6).

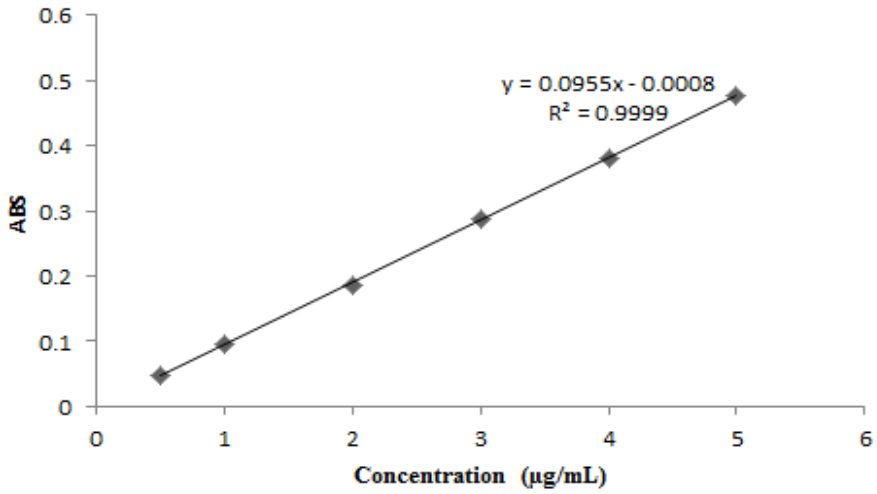

Figure 2. Rutin calibration curve

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The isofraxedin in the concentration range was 30-60 µg/mL, the calibration equation y=0.0653 x + 0.0019, with a regression value of r^2= 0.9990. The RSD was 0.84% (n=4).

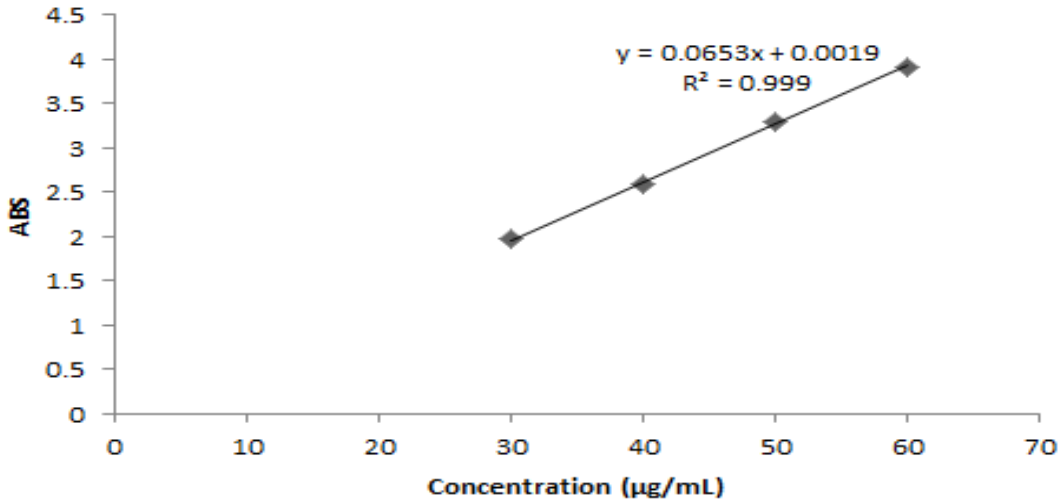

Figure 3. Isofraxedin calibration curve

Also, while for piperine in the range 2-20 µg/mL, the calibration equation y=0.0793 x - 0.0818, regression value of r^2= 0.9962. The RSD was 0.55% (n=6)

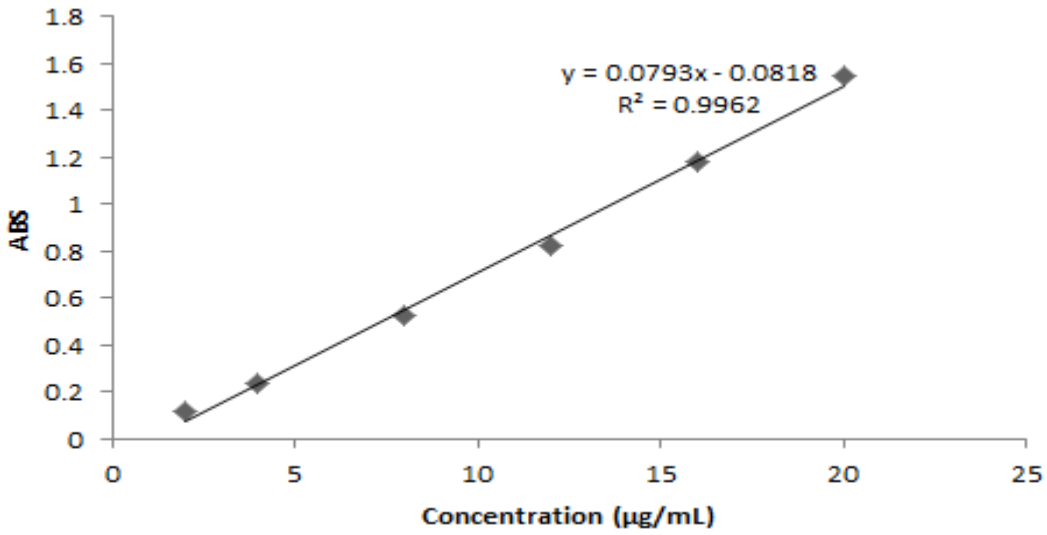

Figure 4. Piperine calibration curve

All the calibration graphs showed strong positive linear correlation (r), which is close to +1. Thus, the content of total phenolic compounds, flavonoids, coumarins and alkaloids in traditional prescription was calculated by the formula of linear equation of the above standard substances were shown in figure 1, 2, 3 and 4 respectively. The results are summarized in table 2.

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Table 2. Contents bio-active substances

№	Biologically active substances	Values obtained (%)	Average (%)
1	Total phenolics	6.58	4.38±1.9
		2.97	
		3.61	
2	Total flavonoids	0.74	0.63±0.17
		0.72	
		0.43	
3	Total coumarins	2.32	2.45±0.28
		2.26	
		2.78	
4	Total alkaloids	0.82	0.83±0.06
		0.90	
		0.77	

The "Lonlunsemberu-13" contained high levels of total phenolic compounds (4.38% ±1.9). The total flavonoids, coumarins and alkaloids content in prescription were found to be 0.63% ±0.17, 2.45% ±0.28 and 0.83% ±0.064 respectively.

Phenolic compounds are believed to have some favorable effects on human health such as lowering of the human low-density lipoprotein, reduction of heart diseases and cancer. Antioxidant compounds include vitamins, carotenoids, flavonoids and phenolic. Among them, phenolic compounds and flavonoids are the most important, and exhibit substantial antioxidant activity.

CONCLUSIONS

It can be concluded from the present study that the "Lonlunsemberu-13" prescription possesses various phytochemicals like total phenolic compounds, flavonoids, coumarins and alkaloids in high quantity. These phytochemicals possess various bioactive properties.

Therefore, it is important to understand the mechanism of action of treatment in relation to these biologically active substances in traditional prescription.

We are now trying to identify and determinate the different phytochemicals from the traditional prescription.

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REFERENCES

1. Anna Pekal., Krystyna Pyrzynska. (2014) Evaluation of Aluminium Complexation reaction for Flavonoid Content Assay. Food Anal. Methods, Springer (2014). DOI:10.1007/s12161-014-9814, 7: pp. 1776-1782.
2. Chimedchagraa Ch., Dagvatseren B., Khishigjargal L., et all. (2015) Reference Book of Control of Traditional Medicinal Substances and Drugs. Ulaanbaatar. G-compress. p. 294.
3. Chinese pharmacopoeia commission Pharmacopoeia of the People's Republic of China. Vol 1: People's Medical Publishing House; 2005.
4. Dagvatseren B., Khishigjargal L., Narantsetseg D., (2014) Reference Book of Traditional Medicines and Herbal Materials. Ulaanbaatar. Admon. P. 64.

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DOI: https://doi.org/10.5564/pmas.v60i2.1356

5. Maitreyi Zaveri, Amit Khandhar, Samir Patel, Archita Patel. Chemistry and pharmacology of Piper longum l. International Journal of Pharmaceutical Sciences Review and Research Volume 5, Issue 1, November-December 2010; Article-010.
6. Medicinal Plants in Mongolia. (2013). World Health Organization Regional Office for Western Pacific.
7. Narendra Kumar Singh., Pramod Kumar et all. (2011) UV-spectrophotometric method development for estimation of piperine in Chitrakadi Vati. Scholars Research Library., ISSN 0975-5071, 3(3): pp. 178-182.
8. Nur Syaida binti Yusof. Phytochemical studies and biological activity of cinnamomum microphyllum. (21962) Bachelor of Science with Honours (Resource Chemistry) 2012. pp. 3-9.
9. Pharmacopoeia of the People's Republic of China. (2010), Chinese pharmacopoeia Commission. China Medical Science Press. Vol 1: pp. 27, 89, 100, 291, 335.
10. Preeti Tiwari. (2014) Phenolic and Flavonoids and Antioxidant Potential of Balarishta Prepared by Traditional and Modern Methods. Asian J. Pharm.Ana.; Vol. 4: Issue 1, pp. 05-10.
11. Tensingh N Baliah., Astalakshmi A. Phytochemical analysis and antibacterial activity of extracts from Terminalia chebula Retz. Int.J.Curr.Microbiol.App.Sci (2014) 3(3): pp.992-999.
12. Trivedi M N et al. Pharmacognostic, phytochemical analysis and antimicrobial activity of two piper species. Pharmacie Globale (IJCP) 2011, 7 (05). pp. 1-5.
13. Wagner H., Bladt S. (1996) Plant drug analysis. A Thin Layer Chromatography atlas. Second edition. Springer-Verlag. Berlin. Pp. 4, 126, 196.
14. Wei Zhao., Yueping Ma., Zhong Yuan. (2012). Optimization of extraction method for the polyphenols from twigs of Cinnamomum cassia. Asian Journal of Traditional Medicines., ISSN 1817-4337, Vol.7 (6): pp. 256-258.